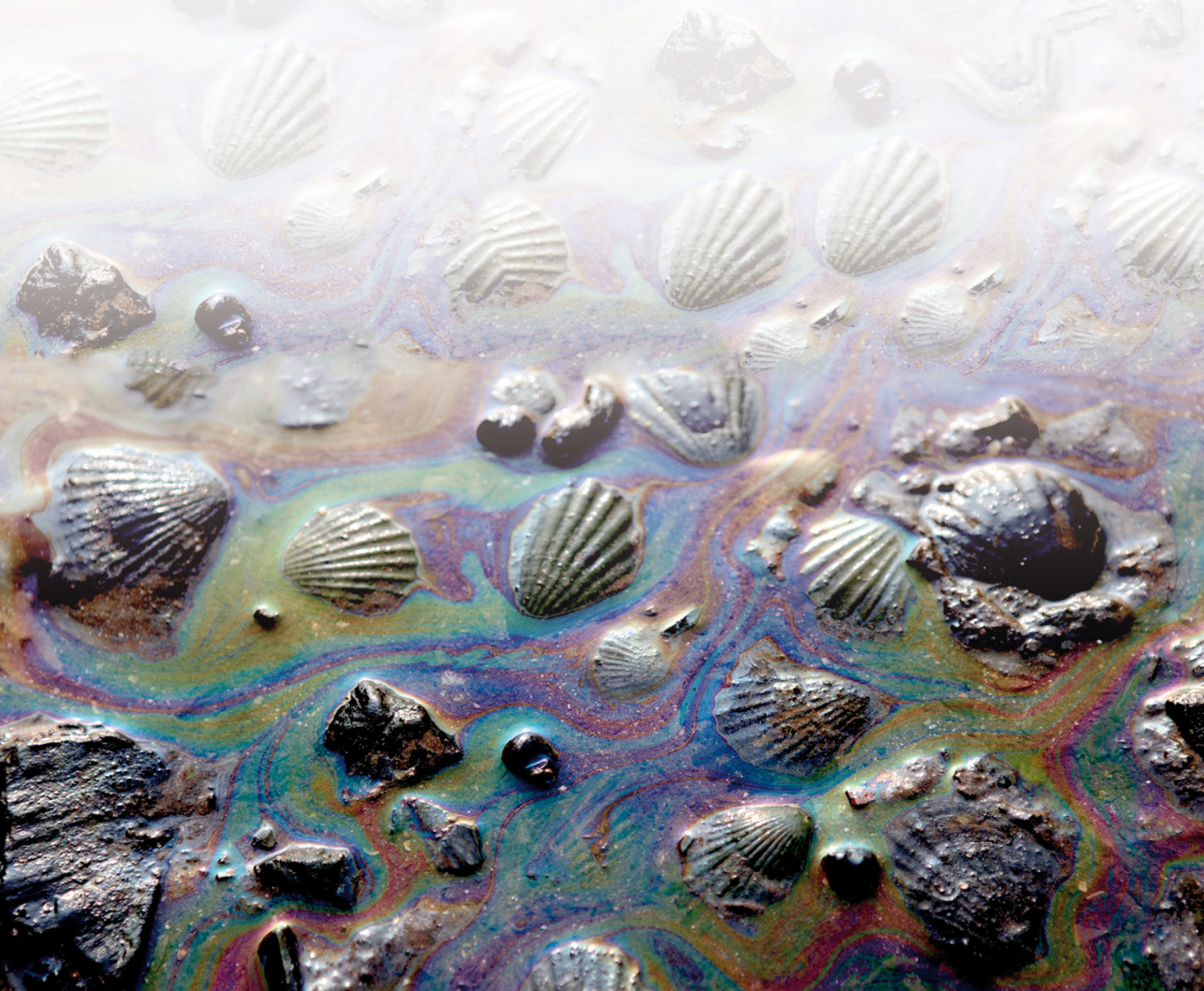
Evaluating chemical exposure and effect models for aquatic species with a focus on crude oil constituents

Lisette de Hoop



De Hoop, L., 2016. Evaluating chemical exposure and effect models for aquatic species with a focus on crude oil constituents. PhD thesis, Radboud University Nijmegen, The Netherlands.

Cover & layout: Roel van den Heuvel
Printed by: Ipskamp Drukkers, Nijmegen

ISBN 978-90-9029883-2

Evaluating chemical exposure and effect models for aquatic species with a focus on crude oil constituents

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus,
volgens besluit van het college van decanen
in het openbaar te verdedigen op
vrijdag 2 september 2016
om 16.30 uur precies

door

Lisette de Hoop

geboren op 22 augustus 1988
te Vlissingen

Promotoren
Prof. dr. ir. A.J. Hendriks
Prof. dr. M.A.J. Huijbregts

Copromotoren
Dr. A.M. Schipper
Dr. F. de Laender (Université de Namur, België)

Manuscriptcommissie
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Paranimfen
Stephanie van Dalen
Thomas van Goethem

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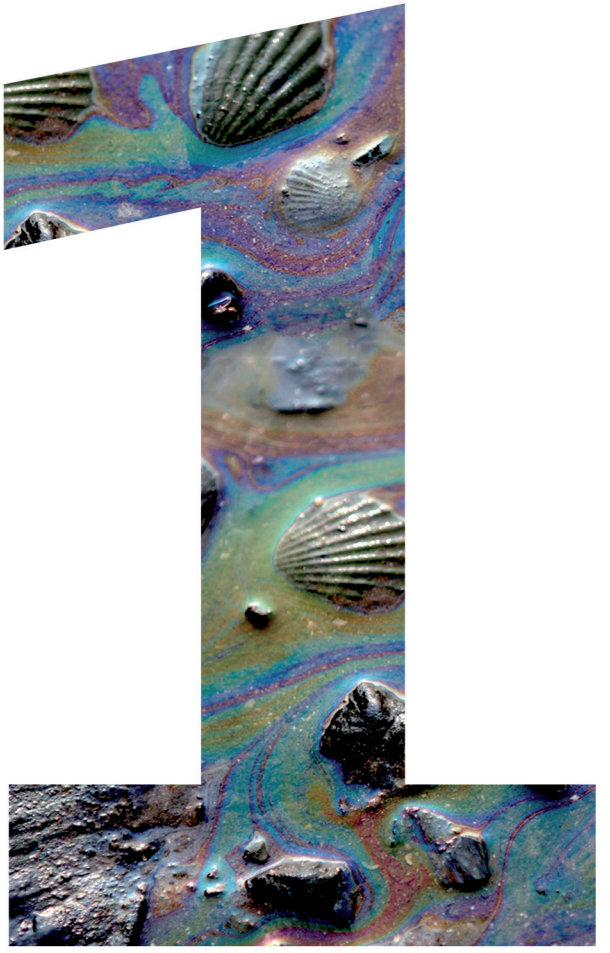


Chapter 1
General introduction

1.1 Ecological risk assessment

1.1.1 General concept

Ecological risk assessment (ERA) aims at predicting the probability of occurrence and the magnitude of adverse effects of chemicals on plants, animals, and ecosystems. Two main factors determine whether a chemical is a potential risk, namely its concentration in the environment and the sensitivity of the organisms exposed, i.e. the chemical dose or concentration that induces an adverse effect, such as lethality (Figure 1.1). The chemical concentration in an environmental compartment, such as water, soil or air, is measured or modelled in the exposure assessment. In the effect assessment, the toxicity of a chemical is determined by establishing concentration-effect relationships. These relationships can be used to derive an environmental concentration at which no effect or a generally acceptable effect occurs, for instance the concentration affecting no more than 5% of the species in an ecosystem. In standard ERA the risk of a chemical is typically characterized by dividing the environmental exposure concentration by the no effect or acceptable effect concentration. If the ratio exceeds a value of 1, the chemical may pose an unacceptable risk to the species or ecosystem of concern.

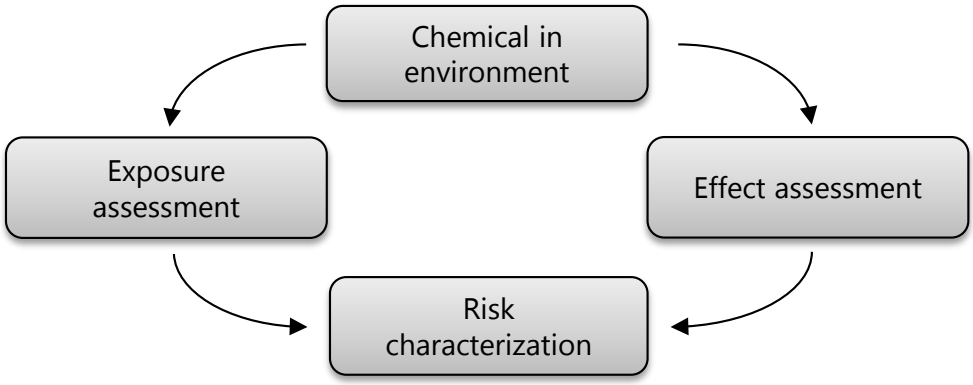


Figure 1.1 The standard risk characterization process for chemical substances (modified after Van Leeuwen and Hermens [1]).

Ecological risks can also be based on internal effect thresholds and body burdens in biota instead of external exposure and effect concentrations of chemicals [2-4]. One advantage of the so-called tissue residue approach (TRA) is that the bioavailability and toxicokinetics (i.e. rates of uptake and elimination) of a chemical are considered. Because internal thresholds are relatively independent of the variability in toxicokinetics, differences in species characteristics are less important in the TRA than in the external concentration approach [4, 5]. Furthermore, internal concentrations integrate exposure over time and space providing a more stable exposure assessment [4]. Yet, limitations to using the

TRA include for instance the assessment of highly biotransformable chemicals, reactive chemicals (e.g. phototoxic chemicals) and inorganics, such as metals [4].

1.1.2 Bioaccumulation

A chemical substance can be taken up by an organism via contact with environmental compartments and via its food. Bioaccumulation occurs when an organism is unable to eliminate all of the absorbed chemical substances. The elimination can include different routes, such as via water, faeces, dilution with biomass and biotransformation of parent compounds into metabolites (Figure 1.2) [6]. Accumulation of a persistent chemical occurs in the fat fraction of the organism if the chemical has a higher affinity to fat compared to water. The potential of organic chemicals to bioaccumulate is therefore often assessed as the octanol-water partition coefficient K_{ow}, which represents the lipophilicity of a chemical and how it thermodynamically partitions between aqueous and lipid phases [7].

The accumulation of a chemical from the water phase can be expressed as a bioconcentration factor (BCF), which is the ratio of the chemical concentration in the organism to the chemical concentration in the water [7]. Body burdens may increase from prey to predator due to dietary absorption, which can be quantified with the biomagnification factor (BMF), that is the ratio of the chemical concentration in an organism to that in the organism's diet [8].

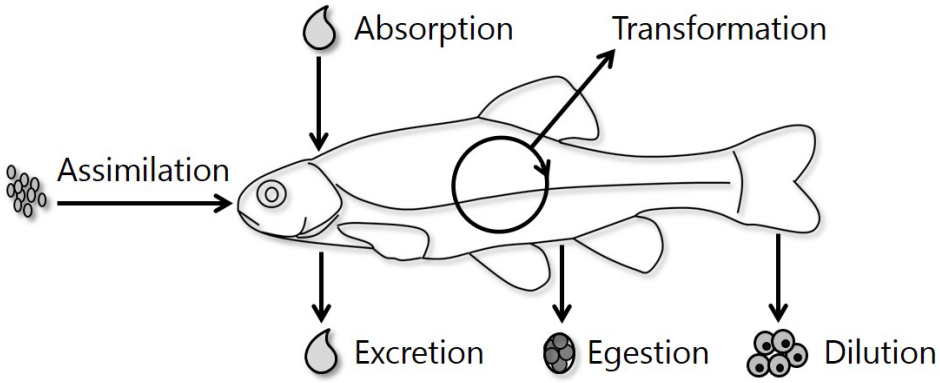


Figure 1.2 Schematic representation of different mechanisms that can carry chemicals into or remove chemicals from aquatic organisms: assimilation from food, absorption from water, transformation in the organism, dilution with biomass, egestion with food, and excretion with water.

Models can be used to estimate the bioaccumulation for organic chemicals and species that have remained untested, in order to limit animal tests and inform regulatory decision making. Relationships between bioconcentration parameters (absorption and elimination rate constants) and the lipophilicity of a chemical substance provide a means for estimating its bioconcentration behaviour [9]. There are different mass-balance models which quantify the bioaccumulation of chemicals in organisms based on the environmental concentration and first-order uptake and elimination rate constants:

1. One-compartment models, in which the body is treated as one unit, since an immediate distribution and equilibrium of the chemical is assumed throughout the organism's body [6, 9]. An example is the bioaccumulation model OMEGA (Optimal Modelling for EcotoxicoloGical Applications), which has been used for a variety of aquatic and terrestrial vertebrates and invertebrates exposed to metals and organic chemicals [10, 11].

2. Multi-compartment models, in which extra compartments are added into which the chemical may distribute. Some models divide the organism into a number of compartments (water compartment, target compartment, and others) with a permanent chemical equilibrium among them [12, 13].

1.1.3 Single-species effects

Accumulation of a chemical can result in an adverse effect on the organism if its body burden exceeds a critical internal threshold level. Concentration-effect relationships can be used to determine a threshold value, such as the critical body burden (CBB), e.g. the internal concentration of a chemical at which 50% of the individuals are negatively affected regarding for instance their growth or survival. Additionally the concentration-effect curves represent the variation in sensitivity between individuals, i.e. the intraspecies variation.

The CBB concept is often used for assessing the potential toxicity of chemicals with a narcotic toxic mode of action (TMoA), i.e. a baseline toxicity [2]. Many organic compounds, like aromatic hydrocarbons, are expected to exhibit a non-polar narcotic TMoA, which is believed to be the result of nonspecific disturbance of membrane integrity and functioning due to partitioning of toxicants into biological membranes [14]. It is assumed that chemicals with the same TMoA exhibit a similar CBB, for example 0.1 (0.04-0.16) mol/kg lipid for narcotic chemicals [5].

1.1.4 Multi-species effects

Multi-species population models are important for ecological risk assessment as effect estimates on a population level usually lie closer to the interest of risk assessors than individual-level effect estimates. The abundance of organisms changes when the demographic vital rates that drive population dynamics,

such as survival and reproduction, are affected. By affecting these vital rates, chemicals subsequently affect population-relevant endpoints, such as the intrinsic growth rate and carrying capacity. These endpoints can be included in a model to simulate the time-varying changes in population biomass and thereby improve the understanding of chemical effects on population dynamics. Interactions between species are also commonly included in population models, such as predator-prey interactions [15, 16]. Multi-species models vary from simple to complex. Consumer-resource population models, such as the Rosenzweig-MacArthur model, describe the interaction between two species only [17]. More complex population models include trophic links between multiple species. These models allow for the identification of possible indirect effects of chemicals via top-down or bottom-up trophic influences due to ecological interactions such as competition [18, 19]. An example of such a more complex model is SINMOD which includes a 3D hydrodynamic-ecological model and a 13-stage structured population model [20].

Another approach to quantify multi-species effects is via species sensitivity distributions (SSD). An SSD is based on toxicity data of multiple species within a region and therefore represents inter-species variation in sensitivity [21]. Whereas dynamic population models usually assess changes in organism abundances over time, an SSD uses static endpoint data, such as LC_{50} values, to quantify the affected fraction of species and the corresponding hazardous concentrations of a chemical for an entire community.

1.2 Aim, focus and outline of the thesis

The aim of this PhD thesis is to evaluate a suite of exposure and effect models on their applicability in ecological risk assessment for aquatic species and ecosystems. The focus is on oil constituents, as it is largely unknown whether current ecological exposure and effect models are applicable to crude oil and its constituents [22]. A general key question underlying the chapters in this thesis is whether we can use simple, generic models to assess the risk of specific chemical pollutants in specific systems, such as oil constituents in aquatic systems. More specifically, the following unknowns in the applicability of exposure and effect models for ERA of organic chemicals are addressed:

1. *Bioaccumulation*: several one-compartment bioaccumulation models for organic chemicals have been developed, but few have been used to estimate the accumulation of oil constituents in aquatic species. The few studies available have focused mainly on the accumulation of polycyclic aromatic hydrocarbons (PAHs) in fish species [9, 23, 24]. Yet, in the event of an oil spill aquatic organisms of various taxonomic groups (not only fish, but also e.g. algae and invertebrates) are exposed to chemicals other than PAHs. Crude oil is a complex mixture of chemicals, which for example also includes cyclohexanes and various straight-chain, ring and branched structures, such as paraffins [25]. Therefore, **chapter 2** analyses the applicability of the OMEGA bioaccumulation model for estimating oil constituent accumulation in cold-blooded aquatic species by comparing model predictions of kinetic rates (absorption and elimination) and BCFs with measured values. The variables water temperature, exposure duration, molecular mass of the oil constituents and biotransformation rates are tested as possible explanations of the performance of the model.

2. *Single-species effects*: the CBB approach has been used in toxicokinetic-toxicodynamic (TKTD) modelling to estimate the time course of lethal effects of a few PAHs on an amphipod species [26]. Other examples of TKTD models are the Damage Assessment Model [27], the Threshold Damage Model [28], and DEBtox [29]. Until now, however, no TKTD model is available to assess the time-varying effects of exposure to a wide range of oil components for a wide range of species. In **chapter 3**, the OMEGA bioaccumulation model is parameterized and tested to quantify the time-varying effects of eight aromatic oil constituents on the survival of crustaceans and fish. The model is based on key parameters applicable to an array of species and compounds with baseline toxicity reflected by a generic CBB. Model estimates are eventually compared with experimental data in chapter 3.

3. *Multi-species population effects*: Complex population models may generate more accurate predictions, yet require more input data than simpler models. This may give rise to difficulties in the parameterization when being used for untested species and chemicals. It has already been suggested to assess

the performance of different types of population models – from simple to complex – in terms of providing outputs relevant for ecological risk assessment [30]. However, until now, it has not been tested how estimates of the effects of crude oil on zooplankton biomass in the Arctic region compare between a complex and a simpler population model. **Chapter 4** describes the difference between the complex multistage model SINMOD and a simpler consumer-resource population model for estimating the effects of crude oil on the (sub) arctic copepod *Calanus finmarchicus*. A relative change in biomass is determined by using a reference scenario and an oil spill scenario.

4. *Consumer-resource population dynamics*: Assessing the interactions between species in addition to direct toxic stressor effects may help to further understand population dynamics. A first step to pinpoint the combined effects of toxicants and species interactions is to include only few species in the assessment. This reduces the amount of factors influencing the system. However, most population models have focused on either single-species or multispecies systems [18, 19, 31]. Until now, the consumer-resource dynamics of two interacting species under contaminant stress has only been studied for pentachlorophenol (pesticide) and nitrogen exposure of rotifers and algae [16, 32, 33]. There is a need for an evaluation of a relatively simple model for estimating (long-term) effects of organic chemicals, thus also chemicals other than oil constituents, on population dynamics of two interacting aquatic species. **Chapter 5** combines short-term experiments with food chain modelling to explore the long-term effects of a toxic stressor on consumer-resource dynamics in a marine intertidal ecosystem. The toxic stressor is the herbicide atrazine in order to assess the indirect effects on the copepod *Delavalia palustris* via its food, the marine diatom *Seminavis robusta*.

5. *Species sensitivity distributions*: so far, regulatory risk procedures and acceptable effect concentrations specific to the marine polar region are lacking for crude oil (constituents). Polar risk assessments are mostly based on toxicity data obtained for temperate species. It has been suggested that toxicity data of temperate organisms are not representative of polar communities due to differences in species sensitivity to contaminants [34]. However, until now, a comparison of polar and temperate marine species regarding their sensitivity to crude oil (constituents) is lacking. **Chapter 6** compares the sensitivities of polar and temperate marine cold-blooded species to crude oil and two oil constituents (2-methyl-naphthalene and naphthalene). SSDs are therefore constructed for polar and temperate species based on toxicity data which comprised acute LC_{50}, EC_{50} and TL_m (median tolerance limit) endpoint values, with mortality or reduced survival effects for 50 percent of the test organisms.

1.2.1 Outline

In Figure 1.3 a schematic overview is given of the different steps in ERA that are addressed in chapters 2-6 of the thesis. Finally, **chapter 7** provides a synthesis of the preceding chapters by discussing the results in the light of the general key question and the assumptions made in this thesis, and general conclusions and recommendations are drawn.

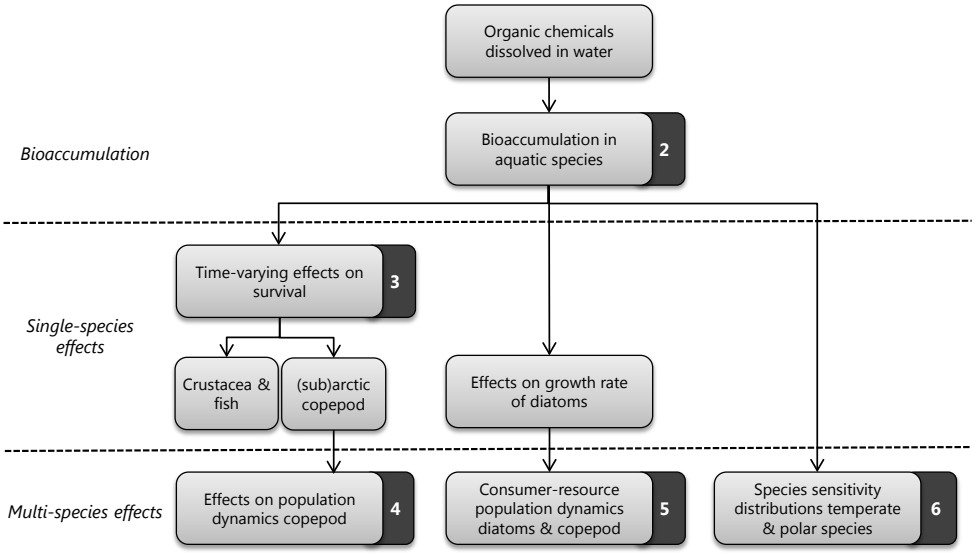
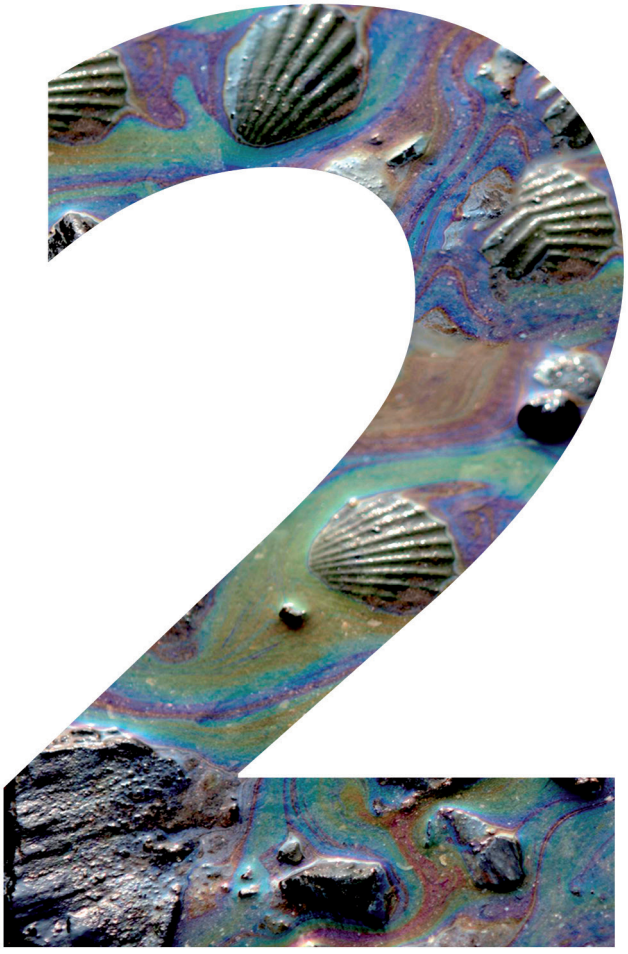


Figure 1.3 Schematic overview of the thesis chapters, covering exposure (Chapter 2), single-species effects (Chapter 3) and multi-species effects (Chapters 4-6).



Chapter 2
Modelling bioaccumulation of oil constituents in aquatic species

Lisette de Hoop
Mark. A.J. Huijbregts
Aafke M. Schipper
Karin Veltman
Frederik de Laender
Karel P.J. Viaene
Chris Klok
A. Jan Hendriks

Published in Marine Pollution Bulletin 76 (1-2): 178-186

Abstract

Crude oil poses a risk to marine ecosystems due to its toxicity and tendency to accumulate in biota. The present study evaluated the applicability of the OMEGA model for estimating oil accumulation in aquatic species by comparing model predictions of kinetic rates (absorption and elimination) and bioconcentration factors (BCF) with measured values. The model was a better predictor than the means of the measurements for absorption and elimination rate constants, but did not outperform the mean measured BCF. Model estimates and measurements differed less than one order of magnitude for 91%, 80% and 61% of the absorption and elimination rates and BCFs of all oil constituents, respectively. Of the "potentially modifying" factors: exposure duration, biotransformation, molecular mass, and water temperature, the last two tended to influence the performance of the model. Inclusion of more explanatory variables in the bioaccumulation model, like the molecular mass, is expected to improve model performance.

2.1 Introduction

Petroleum industry activities may contribute to contamination of marine waters, for example via the discharge of water produced during oil extraction, and accidental spills from shipping and drilling. In the near future, oil exploitation and transportation is expected to increase due to the large energy demand and a changing environment [35]. For instance, the current decline in the extent and thickness of Arctic ice offers opportunities for oil exploitation in hitherto unexplored regions. Simultaneously, oil exploitation might become more risky as the increasing amount of moving, newly formed ice could damage rigs and vessels [36]. More petroleum industry activities will thus increase the risk of oil contamination of marine ecosystems [37].

Since crude oil poses a risk to marine ecosystems due to its toxicity and tendency to accumulate in biota, quantitative information on oil bioaccumulation is important for risk assessment and to establish environmental quality guidelines [37-39]. Risk estimates can be obtained by comparing internal concentrations with a critical internal concentration, the so called critical body burden (CBB), at which detrimental lethal or sublethal effects occur in organisms. Internal concentrations can be derived from measurements and by using bioaccumulation models that can estimate internal concentrations based on kinetic parameters, e.g. uptake and elimination rate constants [23]. The use of models can limit additional animal testing and inform regulatory decision making.

Although several bioaccumulation models have been developed [38], few have been used to quantify the accumulation of oil constituents in aquatic species. The few studies available have focussed mainly on the accumulation of polycyclic aromatic hydrocarbons (PAHs) in fish species [9, 23, 24], whereas species other than fish, such as algae and invertebrates, will be exposed to oil constituents as well. Furthermore, oil is a complex mixture of constituents, including not only PAHs but also various alkylphenols and straight-chain, ring and branched structures, such as paraffins [25]. In the present study, oil accumulation was therefore estimated for aquatic species using the OMEGA bioaccumulation model [6]. In this model absorption and elimination rate constants are quantified as a function of the octanol-water partition coefficient (K_{ow}) of the constituent and the weight, lipid content, and trophic level of the species [6]. These data are relatively easy to obtain. Additionally, several parameter values in the model have been determined with allometric relations. The OMEGA model therefore facilitates bioaccumulation estimations of many chemicals and species, in contrast to most other bioaccumulation models which depend on experimental chemical- and species-specific data. The OMEGA model has been successfully applied to estimate the internal concentrations of metals and several organic pollutants (e.g. biocides, ethers) for various invertebrate and vertebrate species [6, 40-42].

The overall aim of the current study was to evaluate the applicability of the OMEGA model and to explore if the model needed improvements for estimating the accumulation of oil constituents in aquatic organisms. To this end, absorption and elimination rate constants and bioconcentration factors (BCFs) estimated with the OMEGA model were compared with measured values reported in literature for aquatic species from different taxonomic groups (e.g. Crustacea, Mollusca and Osteichthyes) exposed to constituents from different oil groups (i.e. mono-, di-, and polycyclic aromatic hydrocarbons, phenols and n-paraffins). Additionally, differences between the model estimates and measurements were evaluated in relation to water temperature, exposure duration, molecular mass of the oil constituents, and biotransformation rate constants. Finally, model estimates for hydrocarbons were compared with model estimates for other organic compounds (e.g. biocides, ethers) to compare variability among oil constituents with variability among organic compounds in general.

2.2 Methods

2.2.1 Experimental data collection

Laboratory-derived rate constants for oil constituents were collected from publications obtained with the ISI Web of Knowledge and Google Scholar search engines. We used the search terms: 1) oil, petroleum, aromatic, aliphatic, resin, phenol, alkane, alkene, alkyne, paraffin, thiophene, olefin, naphthenic mono- and di-aromatic and 2) elimination, excretion or efflux rate, and uptake, absorption or influx rate. Using the reference lists of papers thus obtained, we searched for additional publications. Our search resulted in 10 papers with 66 absorption and 61 elimination rate constants for 10 aquatic species (crustaceans, fish and molluscs) [43-52]. Additionally, 80 absorption rate constants for 19 aquatic species and 164 elimination rate constants for 29 aquatic species exposed to aromatics and phenols were derived from four studies that used these data for calibration of the OMEGA model [6, 10, 53, 54]. Thus, the data set consisted of kinetic rates found in the literature and of rates used for OMEGA calibration.

To ensure independency, BCF values for oil constituents were searched for in scientific literature sources other than the sources containing absorption and elimination rate constants. In total, 528 BCF values were found for 42 aquatic species (including algae, annelids, crustaceans, diatoms, fish, insects and molluscs) exposed to 26 mono-, di- and polycyclic aromatic hydrocarbons (MAHs, DAHs and PAHs) and n-paraffins in the U.S. EPA Ecotox database [55]. The Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals [56] and The National Library of Medicine's Hazardous Substances Data Bank [57] provided nine studies with oil BCF data for algae, crustaceans, insects, fish and molluscs [58-66]. The ISI Web of Knowledge database provided seven additional studies based on the following search terms: 1) oil, petroleum, aromatic, aliphatic, resin, phenol, alkane, alkene, alkyne, paraffin, thiophene,

olefin, naphthenic mono- and di-aromatic and 2) bioconcentration factor and BCF [23, 67-72].

A contaminant was considered to be an oil constituent when included in the CONCAWE library of the PETROTOX model [73] or when mentioned as such in the literature. Each oil constituent was assigned to one of five oil groups that were considered homogeneous with respect to their chemical structure, i.e. the number of aromatic rings (one, two and more than two, i.e. mono-, di-, and polycyclic aromatics), unbranched hydrocarbons (alkanes i.e. n-paraffins) and hydroxyl groups (phenols) [74]. The number of data did not allow for a more specific classification. The molecular mass of the constituents typically ranged from 78-162, 128-204, 166-280, 94-220 and 170-310 Da for the MAH, DAH, PAH, phenol and n-paraffin groups, respectively, reflecting the number of (carbon) atoms the molecules were composed of. All oil groups included non-alkylated (C0) and alkylated (C1-C3) constituents, except for the n-paraffins.

For comparison, 253 absorption and 551 elimination rate constants and 143 BCFs for, respectively, 22, 57 and 17 aquatic species exposed to persistent organic compounds other than oil, such as biocides and ethers, were derived from four studies that used these data for calibration of the OMEGA model [6, 10, 53, 54].

2.2.2 Data treatment

The BCFs were based on parent and radiolabelled compounds measured in the water and in the whole organism or its organs, such as the liver and bile. BCFs based on species wet weight were divided by the fat fraction of the whole species or the species organs to normalize differences in lipid fractions between species. To include BCFs reported on dry weight as well, the values were converted with a species- specific dry-to-wet weight ratio or a default ratio for the species' taxonomic group (Appendix, Table A1). The geometric mean was used when multiple rate constants or BCF values were available for a single species and single constituent. All absorption and elimination rate constants and BCFs that were collected are available in the Supporting Information which is published online.

2.2.3 Model estimates

The OMEGA bioaccumulation model estimates the internal chemical concentration in an organism based on the uptake and elimination rate constants of the chemical. These rate constants are a function of the chemical property K_{ow} and the species' wet weight, lipid content and trophic level [6]. The current study estimates the absorption of a chemical via the water phase ($k_{0,in}$; μg L/μg kg wet weight·day^{-1}). Elimination from the species can be estimated via water ($k_{0,out}$), faeces ($k_{1,out}$) and dilution by biomass as a consequence of growth or reproduction ($k_{2,out}$). The total elimination rate constant is the sum of these three elimination rate constants ($\Sigma k_{j,out}$; kg/kg·day^{-1}). Although measured total elimination

rate constants can include elimination via biotransformation of chemicals in organisms, this route was not included in the total elimination estimates due to a lack of data for the different taxonomic and oil groups [54]. The steady state BCF (µg L/µg kg lipid weight) is determined as the ratio between the estimated absorption and total elimination rate constants. A conceptual diagram of the OMEGA model, the model equations, parameter values and variables used are available in the Appendix, A1 and Table A2.

To estimate rate constants and BCFs with the OMEGA model, all K_{ow} values of the constituents included in the two empirical data sets were calculated with the KOWWIN model in the EPI Suite programme [75]. The wet weight, lipid fraction, taxonomic classification and trophic level of the species in the data sets were collected from the literature. If weight was not reported in the experimental study, a default adult weight was used, obtained from other studies or estimated from length-to-weight ratios [6]. We assumed all oil constituents to accumulate in the lipid of the organisms. The lipid fractions of species and their tissues were available for 58% of the kinetic rate constants and 17% of the BCFs. These fractions ranged between 0.01-0.12 for fish, 0.003-0.07 for arthropods, 0.01-0.15 for molluscs and 0.01-0.08 for annelids. Default values based on the trophic level of species were used if no fat percentage was reported in the experimental study (i.e. 0.01 for unicellular organisms, 0.03 for annelids, arthropods and molluscs and 0.05 for fish, Table A2).

2.2.4 Evaluating model performance

The absorption and elimination rate constants and BCFs estimated with the OMEGA model were compared with the laboratory-derived values collected from the literature. These measured and estimated data were log-transformed.

First, the coefficient of efficiency E (i.e. the predictive squared correlation coefficient q^2) was calculated, according to:

$$E = 1 - \frac{\sum_{i=1}^{n}(O_i - P_i)^2}{\sum_{i=1}^{n}(O_i - \bar{O})^2} \qquad \text{Equation 2.1}$$

where Oi is the observed value for case *i*, P_i is the estimated value for case *i*, n is the number of cases and $\bar{O}$ denotes the mean of the observed values [76]. E ranges from minus infinity to 1, with a value of 1 indicating perfect model estimation. A positive E indicates that the model estimates rate constants and BCFs more accurately than the average of the observed values.

Second, an absolute error measure was obtained by calculating the average difference between the model estimates and measured values as the root-mean-square-error (RMSE):

$$RMSE = \sqrt{\frac{1}{n} \times \sum_{i=1}^{n}(O_i - P_i)^2} \qquad \text{Equation 2.2}$$

The RMSE summarizes both random error and systematic bias [77].

Next, differences between estimated and measured absorption and elimination rate constants and BCFs were related to the water temperature, exposure duration, molecular mass of the constituent and biotransformation rates (fish taxa only), as these variables were not accounted for in the OMEGA model. A correction factor for temperature dependence of kinetic rate constants was already included in the model, but this multiplication factor was set at 1 due to a lack of experimental data [6]. The exposure duration may be of relevance for BCF estimates, because steady state is not reached instantly. The molecular mass, a chemical property covarying with hydrophobicity (i.e. K_{ow}), the cross section and chain length of a molecule, has been suggested to influence bioaccumulation of organic constituents [78, 79]. Finally, labile constituents may be biotransformed into metabolites that are more water soluble, and thus more susceptible to elimination [80].

Linear regression was applied to assess relationships between model performance and each of the four explanatory variables. Here, we expressed model performance as the ratio between log-transformed estimated and measured rate constants and BCFs, with a positive ratio indicating model overestimates. The geometric mean of rate constants and BCFs was determined prior to applying the linear regression to molecular mass and biotransformation rates. No geometric means were determined for water temperature and exposure duration, since different temperatures and durations were available for a single species and a single constituent. The significance of the linear trends was determined with the Student's t-test.

In literature, measured biotransformation rate data are lacking for most species and chemicals [54]. Estimated biotransformation rate constants for fish were therefore used to evaluate the performance of the model in relation to the biotransformation of chemicals. These rate constants were obtained for oil and non-oil organics from the biotransformation rate constant model in the EPI Suite programme [75]. This model estimates whole body primary rate constants for organic chemicals in a 1 kg fish based on the K_{ow}, the biological half-life and the molecular weight of a chemical (i.e. quantitative structure-activity relationship) [81]. The biotransformation rate constants for 1 kg fish were converted to values corresponding with the actual weight of the fish species by multiplying with $weight^{-\kappa}$, as rate constants scale to organism size with the exponent $-\kappa$ [82].

2.3 Results

Overall, model estimates for absorption ($k_{0,in}$) and elimination rate constants ($\Sigma k_{j,out}$) were more accurate than those for the BCFs of oil constituents. Coefficients of efficiency for absorption and elimination were positive, with E = 0.51 and 0.16 (Table 2.1), and model estimates differed by less than one order of magnitude from the measured data for 91% and 80% of the rate constants, respectively (Figure 2.1). The average differences between the model estimates and measurements were a factor of 3.4 and 7.6 for absorption and elimination rate constants, respectively (factor = $10^{\wedge RMSE}$, with RMSE values of 0.53 and 0.88; Table 2.1). In contrast, the coefficient of efficiency was negative for BCFs (E = -0.20) and model estimates differed by less than one order of magnitude from the measured data for 61% of the values (Figure 2.1).

Table 2.1 The number of data (n), coefficients of efficiency (E) and the root-mean-square-errors (RMSE) of log-transformed absorption and elimination rate constants and bioconcentration factors (BCF) divided into various groups of oil and persistent non-oil organic constituents and taxonomic groups of aquatic species

Groups	Absorption ($k_{0,in}$)			Elimination ($\Sigma k_{j,out}$)			Bioconcentration factor [a]		
	n	E	RMSE	n	E	RMSE	n	E	RMSE
Oil constituents	120	0.51	0.53	165	0.16	0.88	168	-0.20	1.26
Oil groups									
MAHs	2	-	-	3	-0.87	0.65	16	-0.92	0.95
DAHs	12	- 1.45	0.74	20	0.65	0.67	37	-0.06	0.92
PAHs	102	0.62	0.47	135	0.08	0.90	103	-0.49	1.22
Phenols	4	0.53	0.45	7	-3.64	1.16	6	-3.77	1.19
n-Paraffins	-	-	-	-	-	-	6	-4.27	3.11
Taxonomic groups									
Annelida	9	0.42	0.59	14	-0.34	0.43	6	0.64	0.31
Chlorophyta	-	-	-	-	-	-	8	0.07	0.91
Crustacea	39	0.51	0.55	40	0.31	1.08	41	-0.24	0.92
Insecta	4	-3.80	0.56	4	-4.55	0.94	7	-1.11	1.18
Mollusca	35	0.50	0.29	49	-1.63	0.59	33	-0.18	1.25
Osteichthyes [b]	33	-0.53	0.66	58	-2.45	1.00	73	-2.18	1.49
Non-oil organic constituents	156	0.23	0.71	372	0.57	0.64	148	-0.14	1.22

[a] Bioconcentration factors are lipid normalized.
[b] Taxonomic group that includes fish.

Model accuracy of absorption rate constants was high for PAH and phenol oil groups and for annelids, crustaceans and molluscs, based on a positive E (0.42-0.62) (Figures 2.1a and A1, Table 2.1). The uncertainty in the model, i.e. the RMSE, ranged from 0.29 to 0.59. Modelled elimination rate constants were accurate for hydrocarbons with two rings or more (DAHs and PAHs), and for crustaceans (Figures 2.1b and A1, Table 2.1; E = 0.08-0.65 and RMSE = 0.67-1.08). Model estimates of BCFs were accurate for the Annelida and Chlorophyta

groups (E = 0.07-0.64, RMSE = 0.31-0.91), but the E was negative for all oil groups (Figure 2.1c, Table 2.1). For example, the OMEGA model overestimated all six BCFs for *n*-paraffins by two to four orders of magnitude (Figure 2.1c). There were no corresponding uptake or elimination data for *n*-paraffins so it was difficult to determine the sources of error in these model-data comparisons.

On the whole, absorption rate constants were more accurately predicted for oil constituents than for persistent non-oil organic constituents (Figure 2.1a), as indicated by the higher goodness-of-fit measure E and the lower absolute error measure RMSE for oil constituents (E and RMSE were 0.51 and 0.53 for oil and 0.23 and 0.71 for non-oil, respectively; Table 2.1). On average, elimination rate constants were more accurately estimated for non-oil organics (E = 0.57, RMSE = 0.64) than for oil constituents (E = 0.16, RMSE = 0.88; Figure 2.1b). For BCFs, the goodness-of-fit measure showed little difference between oil and non-oil organic constituents (Table 2.1).

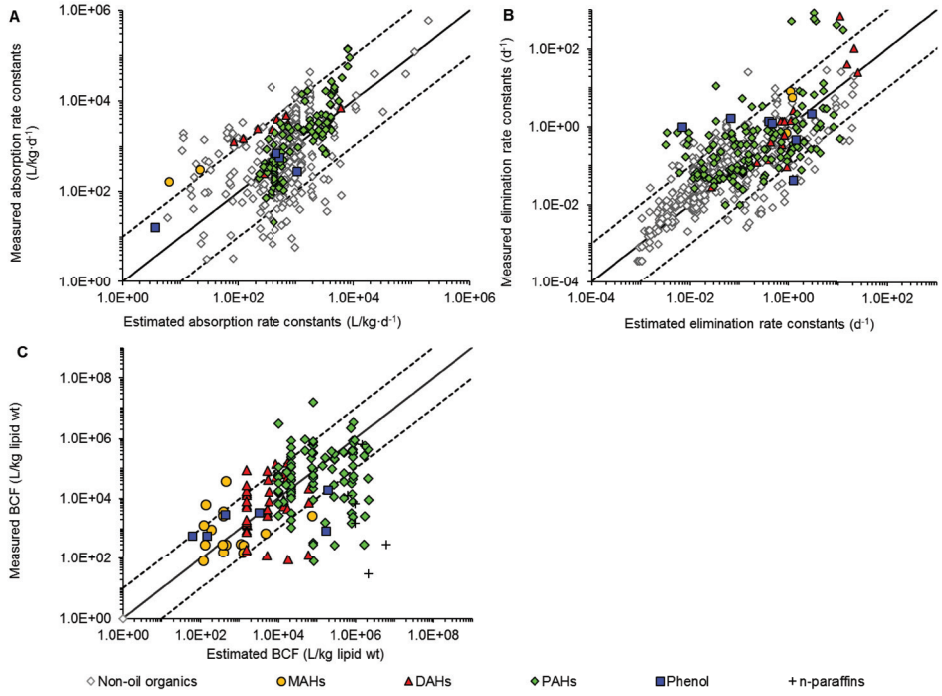


Figure 2.1 Geometric mean for A) absorption rate constants, B) elimination rate constants, and C) lipid-normalized bioconcentration factors (BCF) of organic constituents measured in experiments versus the geometric mean estimated with the OMEGA bioaccumulation model for aquatic species. The coloured diamonds represent different oil groups. The open diamonds represent persistent organic compounds other than oil that have been addressed in previous studies, e.g. biocides, ethers and halobiphenyls [6, 11, 83]. The 1:1 line indicates a perfect model fit. The dashed lines represent a factor of 10 under- and overestimation by OMEGA.

The model performance of BCFs did not show a significant (i.e. $p < 0.05$) trend in relation to water temperature and exposure duration (with and without achieving steady state) (Figures 2.2a and b; Table 2.2). The same held for absorption and elimination rate constants in relation to exposure duration (Figure 2.3, Table 2.3). There was a significant relationship between the model performance of kinetic rate constants and the water temperature (2-30˚C), as the model tended to underestimate both absorption and elimination rate constants at higher temperatures (Figure 2.3, Table 2.3). The performance of the model was also related to the molecular mass of the oil constituents (Figure 2.2c, Table 2.2). On average, the model tended to overestimate absorption rate constants and BCFs for constituents with a molecular mass above 200 Da, whereas elimination rates tended to be underestimated for these constituents (Figure 2.3, Table 2.3). A similar trend was found for BCF model performance and K_{ow}, as 91% of the 43 BCF values were overestimated for oil constituents with a log K_{ow} > 5.5, i.e. PAHs, phenols and paraffins (Appendix, Figure A2). No significant relationship was found between the performance of the model for absorption ($p = 0.99$) and elimination rate constants ($p = 0.86$) and the molecular mass of non-oil organic constituents (Figure 2.3). Finally, the ratio between estimated (excluding biotransformation) and measured (possibly including biotransformation) absorption and elimination rate constants was significantly related to the biotransformation rate constant (Figure 2.3, Table 2.3). The model increasingly overestimated absorption and total elimination rate constants at increasing biotransformation rates. The opposite was found for the BCFs (Figure 2.2d, Table 2.2).

Table 2.2 The coefficient (α), intercept (β), coefficient of determination (R^2), *p*-value and the number of data (n) for the linear regression log(estimated/measured) bioconcentration factor = α · log x + β for four explanatory variables (denoted by x)

Variable (x)	α [a]	β [a]	R^2	*p*-value [b]	n
Temperature (K) [c]	7.12 [-3.92; 18.16]	-17.35 [-44.54; 9.84]	< 0.01	0.21	398
Exposure duration (d) [d]					
- *Steady state*	-0.06 [-0.23; 0.11]	0.18 [0.07; 0.29]	< 0.01	0.49	140
- *Non-steady state*	0.15 [-0.19; 0.50]	-0.39 [-0.57; -0.21]	< 0.01	0.38	100
- *Information on steady state lacking*	-0.29 [-0.44; -0.13]	0.66 [0.49; 0.83]	0.04	< 0.01	351
Molecular mass (Da) [e]	3.84 [2.54; 5.14]	-8.34 [-11.26; -5.43]	0.19	< 0.01	149
Biotransformation rate constants (d^{-1}) [e, f]	-0.94 [-1.42; -0.46]	0.19 [-1.18; 0.56]	0.21	< 0.01	61

[a] The coefficient (α) or intercept (β) and the [lower; upper] 95% confidence interval.
[b] Student's t-test
[c] The water temperature was converted from Celsius to Kelvin prior to log transformation of the data.
[d] The data set was divided according to the information available on chemical steady state.
[e] In case of multiple BCF values for a single species and a single constituent, the geometric mean was determined.
[f] Only fish were included.

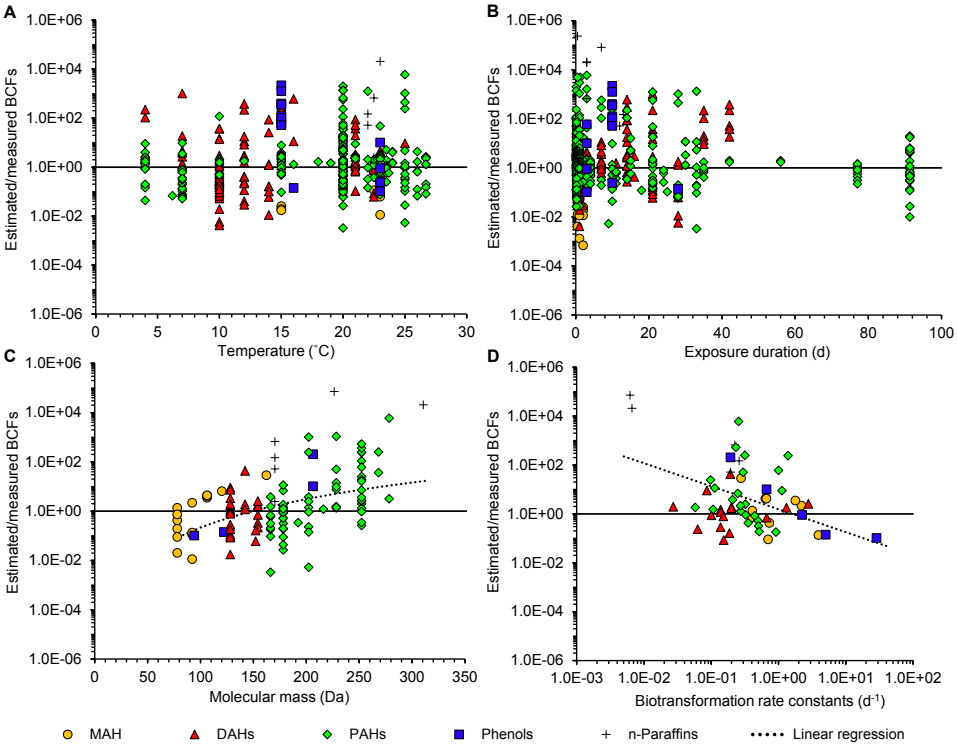


Figure 2.2 Ratio between estimated and measured bioconcentration factors (BCFs) of aquatic species exposed to oil constituents in relation to A) water temperature, B) exposure duration, C) the molecular mass of oil constituents and D) biotransformation rate constants for fish as obtained from the EPI Suite program [75, 81]. The horizontal line indicates a perfect model fit. The dotted lines represent linear regression models (p < 0.05) fitted through all data points of the oil constituents.

Table 2.3 The coefficient (α), intercept (β), coefficient of determination (R2), p-value and the number of data (n) for the linear regression log(estimated/measured) absorption and elimination rate constant = α · log x + β for four explanatory variables (denoted by x)

Rate constant	Variable (x)	α [a]	β [a]	R^2	p-value [b]	n
Absorption	Temperature (K) [c]	-11.33 [-20.66;-2.00]	27.69 [4.69;50.68]	0.05	0.02	104
	Exposure duration (d)	0.10 [-0.02;0.22]	-0.26 [-0.38;-0.14]	0.02	0.11	103
	Molecular mass (Da) [d]	2.27 [1.38;3.16]	-5.35 [-7.40;-3.30]	0.18	< 0.01	119
	Biotransformation rate constant (d^{-1}) [d, e]	0.41 [0.07;0.74]	-0.37 [-0.56;.0.18]	0.16	0.02	33
Elimination	Temperature (K) [c]	-20.01 [-32.79;-7.24]	49.14 [17.67;80.60]	0.06	< 0.01	158
	Exposure duration (d)	0.18 [-0.02;0.38]	-0.26 [-0.44;-0.08]	0.02	0.07	149
	Molecular mass (Da) [d]	-2.36 [-3.60;-1.12]	5.21 [2.36;8.05]	0.08	< 0.01	164
	Biotransformation rate constant (d^{-1}) [d, e]	0.51 [0.17;0.84]	-0.30 [-0.54;-0.07]	0.14	< 0.01	58

[a] The coefficient (α) or intercept (β) and the [lower; upper] 95% confidence interval.
[b] Student's t-test
[c] The temperature was converted from Celsius to Kelvin prior to log transforming the data.
[d] In case of multiple values for a single species and single constituent, the geometric mean was determined.
[e] Only fish were included.

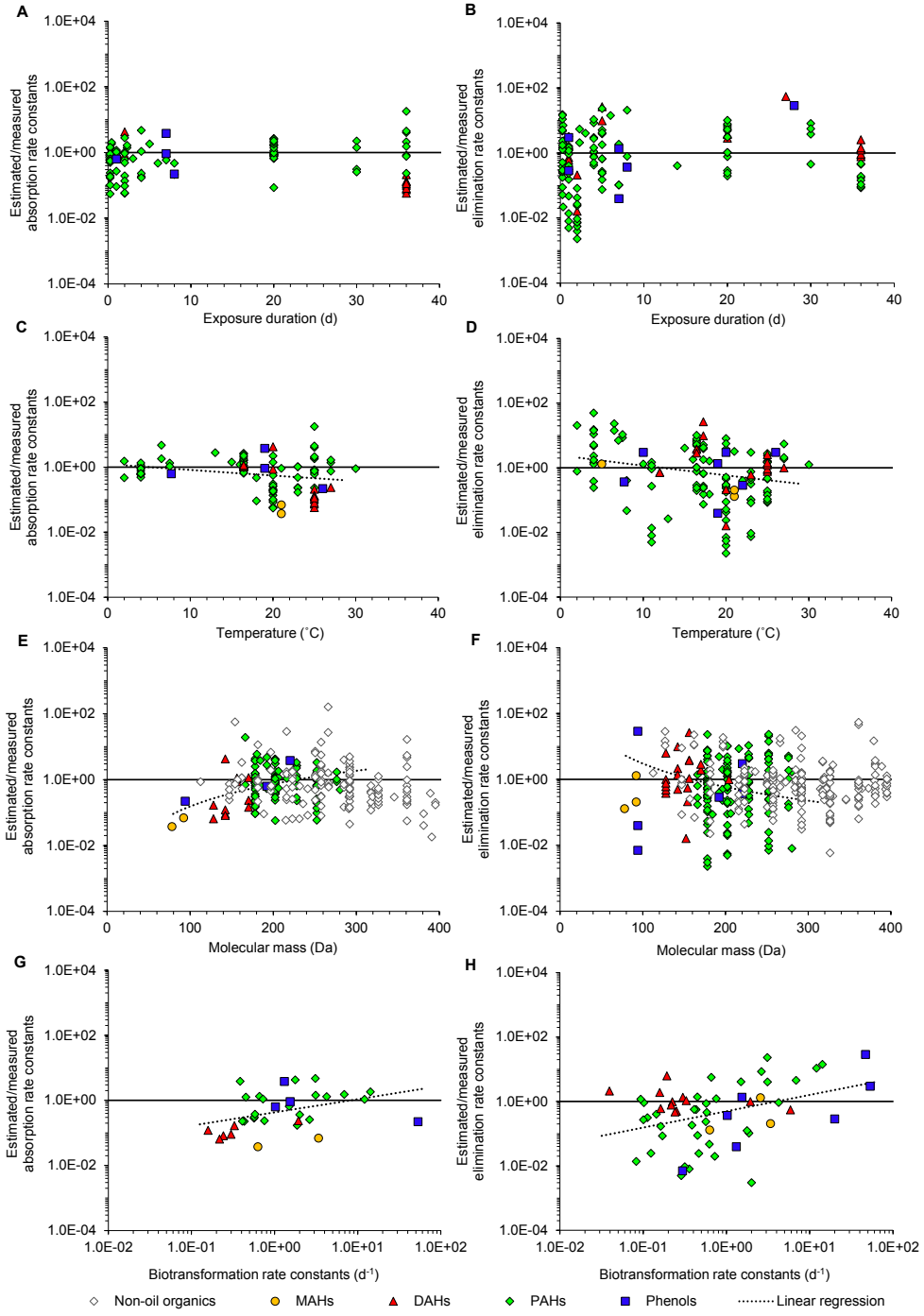


Figure 2.3 Ratio between estimated and measured absorption (A, C, E, G) and elimination (B, D, F, H) rate constants of aquatic species exposed to oil constituents versus exposure duration (A-B), water temperatures (C-D), molecular mass of oil constituents (E-F), and biotransformation rate constants for fish as obtained from the EPI Suite program (G-H) [75, 84]. The horizontal line indicates a perfect model fit. The dotted lines represent linear regression models ($p < 0.05$) fitted through all data points of the oil constituents.

2.4 Discussion

2.4.1 Overall model performance

The OMEGA model estimated absorption and elimination rate constants of all the oil constituents more accurately than the average of the measurements. While for BCFs the average of the measured values was a better predictor than the model. The lower accuracy of the model for the BCFs than for the rate constants may be related to uncertainties in the data set, since the model performance was evaluated with all BCF values that resulted from our literature and database search. Thus, a range of different aquatic species, oil constituents and experimental conditions was included. Yet, no apparent differences were found in the extent of overestimates or underestimates between BCFs based on whole organisms versus organs or between BCFs based on steady state versus non-steady state (Appendix, Figure A3). Additionally, the BCF data (both those determined at steady state and non-steady state) were not related to the exposure duration of the experiments. For this analysis only 250 of the total 611 were used since for these data information on the level of steady state was reported. All the 611 BCFs were used to evaluate the performance of the model for oil constituents in order to cover a wider range of constituents and species. Additionally, measurements on both the radiolabelled and non-radiolabelled oil constituents were included in the analysis. In experiments, radiolabelling might lead to the overestimation of the actual amount of parent compounds present. In the current study, the model underestimated and overestimated experimental BCFs of both the radiolabelled and non-radiolabelled oil constituents (Figure A3). Moreover, the BCF overestimates of radiolabelled constituents were probably biased by fish (Figure A1).

2.4.2 Model performance in relation to water temperature

The performance of the model for BCFs did not show a trend in relation to the water temperature. In contrast, the bioaccumulation model tended to underestimate absorption and elimination rate constants for the higher temperatures. In other words, measured kinetic rates tended to increase slightly with increasing temperature. Yet, these trends explained only 5-6% of the variation in the estimated/measured ratios. Positive trends in relation to water temperature have also been observed for the BCF of hydrophobic organic chemical (HOC) chlorobenzene and the uptake of Bisphenol A in fish [85]. However, the bioaccumulation factors of non-metabolisable HOCs related inversely to temperature, indicating a negative trend between temperature and uptake [85]. Additionally, no trend in relation to water temperature was observed for the elimination rates and BCFs for the oil constituents pyrene and benzo(a)pyrene in the zebra mussel *Dreissena polymorpha* [86]. Based on the current study and literature, relationships between the kinetic rate constants and temperature are ambiguous, requiring in-depth attention in a separate study.

2.4.3 Model performance in relation to molecular mass and biotransformation

Absorption rate constants were less accurately estimated for DAHs than for PAHs. As the molecular mass for DAHs was 128-204 Da compared to 166-280 Da for PAHs, the tendency of the model to underestimate absorption rate constants for oil constituents with a relatively low molecular mass (approximately below 160 Da) might explain the less accurate DAH estimates. Although only two absorption rate constants for MAHs (78 and 92 Da) were available, these rates were also underestimated by the bioaccumulation model (Figure 2.3). The performance of the model for BCFs corresponded with these findings, as BCFs were also underestimated at low molecular mass (Figure 2.2).

Additionally, the 91% overestimated BCFs of oil constituents with a log K_{ow} > 5.5 implied that the performance of the model for relative hydrophobic compounds was influenced by variables not yet included in the model. One of these variables could be the molecular mass, as most BCFs were overestimated above 200 Da. Furthermore, biotransformation might play a role in the performance of the model, for example if the oil constituent is labile and the species has a biotransformation enzyme system. An increased biotransformation will increase the measured total elimination, causing the model to underestimate elimination rate constants and overestimate BCFs. Yet, in the current study a counterintuitive trend was demonstrated by the tendency of the model to overestimate elimination rate constants at high biotransformation rates (Figure 2.3). The causes were unclear for this trend as well as for the remarkable relationship between the biotransformation rates and the performance of the model for absorption rates. The relationship between the model performance of BCFs and the biotransformation rate constants (Figure 2.2) was highly dependent on two highly overestimated BCF values, namely for the n-paraffins docosane and hexadecane. After removal of these two values, the performance of the model for BCFs was no longer related to the biotransformation rate constants. More measured kinetic rate constants and BCFs are needed for an extensive evaluation of the performance of the model for oil constituents in relation to (measured) biotransformation rate constants.

In addition to linear regression, we used another method to test the accuracy of the model for oil in relation to biotransformation rates. Biotransformation by fish was added as a fourth elimination route to the OMEGA model by adding the obtained rate constants from the EPI Suite programme to the estimated total elimination rate constant $\Sigma k_{j,out}$. The modelled elimination rate constants (including biotransformation) were compared with measured elimination rate constants (possibly including biotransformation). Although the coefficient of efficiency E remained negative, the E increased from -2.45 to -1.25 (Table A3). Model performance particularly improved for elimination rate constants of PAHs (E: -6.57 to -2.21, Figure A4), which is consistent with previous studies that showed fish to biotransform PAHs, such as benzo(a)pyrene, fluoranthene and benzo(a)anthracene [87, 88]. The performance of the model for BCFs improved

correspondingly after incorporating biotransformation rate constants in the model. The goodness-of-fit E increased from -2.10 to -0.34 for all oil constituents (Table A4). It may be concluded that in general a minor improvement will be achieved from incorporating biotransformation rates to the model, but the model accuracy can increase for fish exposed to PAHs.

Estimates of elimination rate constants were more accurate for molluscs than for fish, but the model performed best for crustaceans. Differences among species in the ability to biotransform hydrocarbons may contribute to these differences in the model performance between taxonomic groups. For example, the biotransformation rates of several PAHs were reported to be higher in vertebrates (e.g. fish) than invertebrates (e.g. molluscs and crustaceans) [87, 88], which may explain the more severely underestimated elimination rates for fish.

2.4.4 Implications and recommendations

The OMEGA bioaccumulation model predicted absorption and elimination rate constants for aquatic species exposed to oil constituents with an accuracy that is consistent with other bioaccumulation models that focus on chemicals other than oil constituents [38, 89, 90]. Inclusion of more explanatory variables in the bioaccumulation model can improve the performance of the model. Firstly, correcting absorption rate constants for molecular mass may improve model performances for oil constituents with a relatively low mass (e.g. MAHs and DAHs). The molecular mass could be added as a variable influencing the lipid layer permeation resistance. For example, Gobas and Opperhuizen (1986) related the lipid layer permeation rate to the solute's membrane-water partition coefficient and to factors affecting the diffusion coefficient in the membrane layer, such as the molecular mass [9]. In addition to molecular mass, the molecular cross section and chain length have also been suggested to influence the bioaccumulation of organic constituents [78, 79]. In general, a diameter above 0.95 nm and a chain longer than approximately 4.3 nm may cause a decreased membrane permeation of organic chemicals [78]. The majority of crude oils contain straight-chained hydrocarbons (i.e. n-paraffins) that can be up to 35 carbon atoms long [25]. Secondly, relating the absorption and elimination rate constants to the water temperature may slightly improve the kinetic rate estimates. Thirdly, biotransformation could be added as an additional elimination route when estimating the bioaccumulation of oil constituents. This will probably improve model estimations for taxonomic groups that are able to biotransform labile constituents, as shown in the current study for fish exposed to PAHs.

In the future, model performances could be evaluated in relation to these physico-chemical properties if more empirical data become available on for instance n-paraffins and other aliphatics. In the current study, the general applicability of the model to oil constituents and the influence of variables on the model were evaluated with all kinetic rate constants and BCFs resulting from our literature and database search. Alternatively, the performance can be evaluated

by simulating the kinetics of one chemical in one species and comparing it to experimental data. Afterwards, a sensitivity analysis may be carried out to identify the parameters that require most attention.

2.5 Acknowledgements

Frederik De Laender is a postdoctoral research fellow from the Fund for Scientific Research (FWO, Flanders). We thank Chiel Jonker, Eric Verbruggen, Aaron Redman and Duncan King for their suggestions and sharing their knowledge on oil accumulation. We thank the Norwegian Research Council (NRC) for support through the PETROMAKS program (BIP project #ES468602). The SYMBIOSES project is a cooperation of 15 research partners, financed by the NRC, BP Exploration Operating Company Limited, ConocoPhillips Skandinavia, ExxonMobil Upstream Research Company, Eni Norway, Shell Technology Norway, Statoil Petroleum and Total E&P Norway.

Chapter 3

Time-varying effects of aromatic oil constituents on the survival of aquatic species: deviations between model estimates and observations

Lisette de Hoop
Karel P.J. Viaene
Aafke M. Schipper
Mark A.J. Huijbregts
Frederik de Laender
A. Jan Hendriks

Accepted in Environmental Toxicology and Chemistry

Abstract

There is a need to study the time course of toxic chemical effects on organisms, because there might be a time lag between the onset of chemical exposure and the corresponding adverse effects. For aquatic organisms, crude oil and oil constituents originating from either natural seeps or human activities, can be relevant case-studies. In the current study we tested a generic toxicokinetic model to quantify the time-varying effects of various oil constituents on the survival of aquatic organisms. The model is based on key parameters applicable to an array of species and compounds with baseline toxicity reflected by a generic, internal toxicity threshold or critical body burden (CBB). We compared model estimates with experimental data on the effects of eight aromatic oil constituents on the survival of aquatic species including crustaceans and fish. The average model uncertainty, expressed as the root-mean-square-error, was 0.25 (minimum-maximum: 0.04-0.67) on a scale between 0 and 1. The estimated survival was generally lower than the measured survival right after the onset of oil constituent exposure. In contrast, the model underestimated the maximum mortality for crustaceans and fish observed in the laboratory. Thus, the model based on the CBB concept failed to adequately predict the lethal effects of the oil constituents on crustaceans and fish. Possible explanations for the deviations between model estimates and observations may include incorrect assumptions regarding a constant lethal body burden (LBB), the absence of biotransformation products, and the steady-state of aromatic hydrocarbon concentrations in organisms. Clearly, a more complex model approach than the generic model used in this study is needed to predict toxicity dynamics of narcotic chemicals.

3.1 Introduction

Crude oil can be introduced into the aquatic environment via natural seeps and due to human activities like oil extraction, transportation and consumption [91]. Oil drilling activities lead to discharge of water contaminated with oil constituents and added process chemicals. Furthermore, accidents during shipping and drilling can cause the release of large amounts of crude oil to the environment, resulting in mass mortality of aquatic organisms due to physical contamination and oil toxicity [92]. This has been demonstrated by the immediate mortality of crustaceans, fish and mammals after oil spills, for example from the super tanker Amoco Cadiz and the Deepwater Horizon oil rig [93, 94].

Oil has the tendency to accumulate in biota [95]. Microcosm and laboratory studies allow for the examination of oil effects on aquatic species. While the number of experiments has increased over the last decade [29, 92, 96-101], effect data of oil constituents are still lacking for a large number of marine and freshwater species. Lethal effects on individuals, measured in single-species toxicity experiments for a selection of species and chemicals, can be used in mechanistic models to estimate effects on survival for oil substances and species that have remained untested. Various models simulate the time course of toxic effects on organisms by translating external concentrations to internal concentrations and subsequently linking these internal concentrations to effects on organisms [97]. In particular, the Critical Body Residue model and Damage Assessment Model have been used to estimate the time course of toxic effects (LR_{50}: residue at 50% mortality) of a few polycyclic aromatic hydrocarbons (PAHs) in two amphipods and a midge [26, 27]. The Critical Body Residue (CBR) or Critical Body Burden (CBB) concept assumes an immediate adverse effect of a chemical on an organism if an internal concentration threshold is exceeded. Because the toxicity threshold for a given species is assumed invariant, variability in response is attributed to toxicokinetics [102]. DEBtox, a toxicokinetic-toxicodynamic (TKTD) model that simulates energy budgets in organisms and uses a time dependent damage variable, has been used to estimate effects of the oil constituents fluoranthene and pyrene on the survival and reproduction of the water flea *Daphnia magna* [29]. DEBtox uses an internal no-effect concentration (NEC) and a tolerance concentration to relate a metabolic parameter to the body burden in an organism [29].

In TKTD modelling, there is a trade-off between the level of detail and the number of parameters that need to be estimated from experimental data [103]. Application of species-specific and substance-specific models may generate accurate predictions, yet require more input data, which may give rise to difficulties in the parameterization when being used for untested species and chemicals. By contrast, the OMEGA model represents a modelling approach based on relatively few and easily retrievable chemical properties and biological traits, such as the chemical's octanol-water partition coefficient (K_{ow}) and the species' body weight [6, 104]. The model has been successfully applied to estimate the time-varying

population development of copepod (*Eurytemora affinis*) and white-tailed eagle (*Haliaeetus albicilla*) populations exposed to metals and organic pollutants (PCB and DDE), respectively [105, 106]. However, these applications were based on substance-specific toxicity threshold values (EC_{50} and LC_{50}). It has not yet been evaluated whether the OMEGA model can be profitably used to assess toxic effects of oil constituents based on a generic, internal toxicity threshold or CBB.

The main goal of the current study was to parameterize and test the CBB-based OMEGA model to quantify the time-varying effects of oil constituents on the survival of aquatic organisms. First, we estimated the body burden (BB) of oil constituents in aquatic organisms over time [6, 107]. Next, we assumed survival to be a log-logistic function of the body burden in order to estimate the toxic impact of oil constituents on aquatic organisms [31, 108]. For parameterization of the model equations, we used generic values where applicable and chemical- or species-specific data where needed. Finally, the model results were compared with measured effects of eight selected oil constituents (monocyclic, dicyclic and polycyclic aromatic hydrocarbons) on the survival of crustaceans and fish. While the equations should be applicable for different exposure scenarios, we tested the model for constant exposure only because 1) its validity for simple cases should be known before proceeding to complex situations and 2) experiments with variable oil concentrations have not been carried out yet.

3.2 Methods

3.2.1 Model equations

Bioaccumulation. The OMEGA bioaccumulation model [6] estimates the body burden in an organism (i.e. internal chemical concentration) based on the uptake and elimination rate constants of the chemical. These rate constants are quantified as a function of the octanol-water partition coefficient (K_{ow}) of the chemical and the organism's wet weight, lipid content and trophic level [6]. In the current study we estimated the absorption of an oil constituent via the water phase ($k_{0,in}$; L/kg wet weight·day^{-1}). Uptake via food or oil droplets was assumed negligible [109]. Elimination from the organism was assumed to occur via water ($k_{0,out}$), faeces ($k_{1,out}$), dilution by biomass as a consequence of growth or reproduction ($k_{2,out}$) and biotransformation of the chemical ($k_{3,out}$). The total elimination rate constant was the sum of these four elimination rate constants ($\Sigma k_{j,out}$; kg wet weight/kg wet weight·day^{-1}, i.e. day^{-1}). The model did not include the possible body burdens of products formed by biotransformation. Assuming first order kinetics, the time-varying concentration of a chemical c in an organism of species level s (µg/kg wet weight) was calculated as [6]:

$$\frac{dBB_{s,c}}{dt} = k_{0,in} \times C_{w,c} - \sum_{j=0}^{j=3} k_{j,out} \times BB_{s,c} \qquad \text{Equation 3.1}$$

which represents the absorption from water with exposure concentration $C_{w,c}$

(µg/L) and the elimination from the organism with a chemical residue $BB_{s,c}$ (µg/kg wet weight). A conceptual diagram of the OMEGA model can be found in Chapter 2 (Appendix A1) and the model equations used to determine $k_{0,in}$ and $\Sigma k_{j,out}$ are available in Table 3.1.

Effects on survival. The effects of oil constituents on the survival of aquatic organisms were calculated relative to the survival representative of a control situation (no unit; Eqn. 3.2). We assumed the effects to be a logistic function of the estimated body burden [31, 110]:

$$Fraction\ survival_t = \frac{1}{1+(\frac{\max\ BB_{S,c,t}}{LBB})^{slope}} \qquad \text{Equation 3.2}$$

where $\max BB_{s,c,t}$ is the highest body burden that occurred until time t (mmol/kg lipid), LBB the lethal body burden (mmol/kg lipid), i.e. the critical body burden, and the slope represents the inter-individual variation in LBB as represented by the corresponding concentration-response curve [111]. The model assumed an individual tolerance distribution (IT) meaning that individuals die at different body burdens, because they are assumed to have different sensitivities to chemicals [97]. Furthermore, consistent with the CBB concept, death occurs immediately if the lethal body burden is exceeded and the model assumes no effect of a chemical on the metabolic processes of the organisms. The estimated body burden ($BB_{s,c,t}$) was converted from µg/kg wet weight to mmol/kg lipid weight with the molar mass (g/mol) of the oil constituent and the lipid fraction of the organism.

Table 3.1 Generic parameter values and variables used for estimating the effect of oil constituents on the survival of aquatic species

Symbol	Description	Unit [a]	Typical value/Calculated from	Ref
Kinetics (Eqn. 3.1)				
i	Trophic level [b]		1 = algae and plants; 2 = herbivores; 3 = carnivores	
j	Medium		0 = water, 1 = food, 2 = biomass	c
$k_{0,in}$	Absorption rate constant	L/kg·day^{-1}	$\frac{w^{-\kappa}}{\rho_{H_2O,0}+\frac{\rho_{CH2,i}}{K_{OW}}+\frac{1}{\gamma_0}}$	c
$k_{0,out}$	Excretion rate constant	day^{-1}	$\frac{1}{p_{CH2,i}\times(K_{ow}-1)+1}\times\frac{w^{-\kappa}}{\rho_{H2O,0}+\frac{\rho_{CH2,i}}{K_{OW}}+\frac{1}{\gamma_0}}$	c
$k_{1,out}$	Egestion rate constant	day^{-1}	$\frac{1}{p_{CH2,i}\times(K_{ow}-1)+1}\times\frac{w^{-\kappa}}{\rho_{H2O,1}+\frac{\rho_{CH2,i}}{q_T\times K_{OW}}+\frac{1}{p_{CH2,i-1}\times K_{OW}\times(1-p_1)\times q_T\times\gamma_1}}$	c
$k_{2,out}$	Dilution rate constant	day^{-1}	$q_T\times\gamma_2\times w^{-\kappa}$	c
$K_{3,\ out}$	Biotransformation rate	day^{-1}	QSAR for fish	d
$C_{w,c}$	Concentration in water	µg/L	Variable	e
$BB_{s,c}$	Concentration in organism	µg/kg	Variable	c
K_{ow}	Octanol-water partitioning coefficient	-	Variable	f
w	Species body weight	Kg	Variable	g
$p_{CH2,i}$	Lipid fraction of species	kg·kg^{-1}	Default: 0.01 (i = 1), 0.03 (i =2), or 0.05 (i = 5)	h, i
$p_{CH2,i-1}$	Lipid fraction of food	kg·kg^{-1}	Trophic level: 1 = 0, 2 = 0.01, 3 = 0.03	i
κ	Rate exponent		0.25	c
$\rho_{H2O,j}$	Water layer diffusion resistance	day·kg$^{-\kappa}$	$2.8\cdot10^{-3}$ (j = 0), $1.1\cdot10^{-5}$ (j = 1)	c
$\rho_{CH2,i}$	Lipid layer permeation resistance	day·kg$^{-\kappa}$	$4.6\cdot10^{3}$ (i = 1), $6.8\cdot10^{1}$ (i ≥ 2)	c
$p_{1,i}$	Fraction ingested food assimilated	kg·kg^{-1}	0 (i = 1), 0.4 (i = 2); 0.8 (i = 3)	c
q_T	Temperature correction factor	kg·kg^{-1}	1 (cold-blooded organisms)	c
γ_0	Water absorption-excretion coefficient	kg$^{\kappa}$·day^{-1}	200 (water breathing organisms)	c
$\gamma_{1,i}$	Food ingestion coefficient	kg$^{\kappa}$·day^{-1}	0 (i = 1), $5.0\cdot10^{-3}$ (i ≥ 2)	c
γ_2	Biomass (re)production coefficient	kg$^{\kappa}$·day^{-1}	$6.0\cdot10^{-4}$ (All organisms)	c
Dynamics (Eqn. 3.2)				
LBB	Lethal body burden	mmol/kg lipid wt	65.6 (min-max: 12.3-280.0; n = 95)	j
Slope	Slope of concentration-response curve	-	3.0 (min-max: 0.9-24.9 n = 16)	j

[a] Kg is in wet weight;
[b] Crustaceans are considered herbivores; fish are considered carnivores;
[c] [6];
[d] [75, 81];
[e] see Table B4 in the appendix;
[f] [75, 112];
[g] see Table B1 in the Appendix;
[h] [104]; i [113];
[j] see Table B3 in the Appendix.

3.2.2 Model input and parameters

Bioaccumulation. We parameterized the model with generic data where applicable (e.g. the allometric regression exponent) and chemical- or species-specific data where needed (e.g. K_{ow}, species' body weight) (Table 3.1). In order to facilitate comparison of the model outcomes with experimental data from survival experiments (see further section 2.3), we used the oil constituent concentrations in water ($C_{w,c}$) as well as the wet weight and lipid content of the species from the survival experiments themselves. In most experiments a nominal $C_{w,c}$ was reported, except for *P. promelas* and *H. azteca* exposed to pyrene and fluorene [97, 98]. In five out of the six survival experiments the test solutions were changed daily or every other day to achieve the initial concentration specified [27, 29, 97, 98, 114]. If weight or lipid content were not reported, we used a value obtained from other experimental studies on the same species of a similar developmental stage (Table B1, Appendix B). Lipid fractions reported on a dry weight basis were converted with a default dry-to-wet weight ratio for the species' taxonomic group [82]. If no measured lipid fraction could be obtained, we used default values specific to the species' trophic level (Table 3.1). The molecular weight and Kow of the oil constituents were obtained from the CONCAWE database as compiled in the PETROTOX model (Table 3.2; [112]). Data needed to calculate the absorption ($k_{0,in}$) and elimination ($k_{0,out}$, $k_{1,out}$, $k_{2,out}$) rate constants were obtained from the literature [6]. Biotransformation rate data ($k_{3,out}$) were not available for most invertebrate species and oil constituents, except for *Hyalella azteca* and *Pandalus platyceros* exposed to fluoranthene and benzo(a)pyrene, respectively [87, 107, 115]. We therefore did not include biotransformation rate constants for crustaceans. For fish, whole body primary biotransformation rate constants for oil constituents were estimated using quantitative structure-activity relationships (QSARs) based on the K_{ow}, biological half-life and molecular weight of a chemical [75, 81, 107]. Table 3.2 shows an overview of the estimated absorption and elimination rate constants per oil constituent.

Table 3.2 Estimated absorption rates ($k_{0,in}$) and elimination rates via water ($k_{0,out}$), faeces ($k_{1,out}$), dilution by biomass ($k_{2,out}$), and biotransformation ($k_{3,out}$) for several oil constituents in crustaceans and fish

Species	Chemical	K_{ow}	Molar mass (g/mol)	$k_{0,in}$	$k_{0,out}$	$k_{1,out}$	$k_{2,out}$	$k_{3,out}$
Crustacea								
Chironomus tentans	Fluoranthene	$10^{\wedge 5.25}$	202.3	2353.3	1.04	0.05	0.01	
Daphnia magna	Pyrene	$10^{\wedge 5.18}$	202.3	4283.8	0.95	0.04	0.02	
Daphnia magna	Fluoranthene	$10^{\wedge 5.25}$	202.3	4320.1	0.81	0.04	0.02	
Diporeia spp.	Fluoranthene	$10^{\wedge 5.25}$	202.3	2787.2	0.26	0.01	0.01	
Hyalella azteca	Fluoranthene	$10^{\wedge 5.25}$	202.3	2671.2	0.61	0.03	0.01	
Hyalella azteca	Fluorene	$10^{\wedge 4.05}$	166.2	1583.7	5.67	0.03	0.01	
Hyalella azteca	Pyrene	$10^{\wedge 5.18}$	202.3	2648.7	0.72	0.03	0.01	
Fish								
Clupea pallasii	Benzene	$10^{\wedge 2.00}$	78.1	78.7	13.14	0.03	0.03	7.64
Oncorhynchus mykiss	Phenanthrene	$10^{\wedge 4.65}$	178.2	441.5	0.20	0.00	0.00	0.35
Oncorhynchus mykiss	Retene	$10^{\wedge 6.24}$	234.3	524.4	0.01	0.00	0.00	0.28
Pimephales promelas	Trimethylbenzene	$10^{\wedge 3.42}$	120.2	182.7	1.38	0.00	0.00	0.87
Pimephales promelas	Naphthalene	$10^{\wedge 3.35}$	128.2	160.5	1.43	0.00	0.00	0.30

Effects on survival. For the parameterization of Eqn. 3.2, we collected toxicity data from literature pertaining to chemicals with a narcotic toxic mode of action (TMoA) and aquatic species. A narcotic TMoA is believed to be the result of nonspecific disturbance of membrane integrity and functioning due to partitioning of toxicants into biological membranes [14, 116]. The majority of oil constituents are expected to exhibit this so called baseline toxicity based on their chemical structure consisting mainly of carbon and hydrogen [117]. In a previous study, measured mean lethal effect concentrations (HC_{50}) for aquatic species corresponded well with estimated lethal effect concentrations (LC_{50}) expected from a narcotic TMoA for the oil components naphthalene and 2-methyl-naphthalene [37]. In the present study, we therefore parameterized the model with a generic LBB and slope based on internal concentration-response curves pertaining to multiple narcotic chemicals, including oil constituents, and aquatic species.

We determined a geometric mean LBB of 66 mmol/kg lipid (min-max: 12-280 mmol/kg lipid weight) based on 11 aquatic species exposed to chemicals with an expected narcotic TMoA, such as PAHs and fluoro-, chloro-, and bromobenzenes (Tables 3.1 and B2). Most scientific publications do not report the slopes of concentration-response curves [97]. We therefore calculated slopes ourselves by fitting concentration-response functions to the reported raw internal concentration-response data (in mmol/kg lipid weight and percentage survival). An arithmetic mean slope of 3.0 was determined based on narcotic chemicals, such as PAHs, bromobenzenes, chloroethanes, and chlorobiphenyls, affecting the survival of a midge, amphipods and fish (Tables 3.1 and B2). An overview of the LBBs and slopes of concentration-response curves and the corresponding chemicals and species are shown in Tables B2 and B3.

3.2.3 Comparison with experimental data

We compared our model estimates on survival with experimental data on the survival of four arthropod species (Branchiopoda and Malacostraca) and three fish species (Actinopterygii) exposed to various oil constituents, namely pyrene, fluoranthene, fluorene, phenanthrene, retene (i.e. PAHs), naphthalene, and two benzenes (Tables 3.2 and B4) [27, 29, 97, 98, 114, 118]. The experimental survival data were relative to the survival representative of the control situation. One of these studies reported the measured BBs in addition to the measured effect on the survival of an aquatic species [27]. This enabled us to compare estimated and measured BBs in order to separately evaluate the performance of the kinetic part of the model. The experimental data used for comparison were reported averages of the BBs and effects on survival measured in multiple replicates per experimental treatment. None of the experimental studies reported the variability in measurements between the replicas.

3.2.4 Model performance statistics

We calculated the root-mean-square-error (RMSE) to evaluate the overall goodness-of-fit of the model [119]. The RMSE is a relative measure for the performance of the model. First, we calculated the RMSE per species, chemical and exposure concentration:

$$RMSE_{s,c,cw} = \sqrt{\frac{\sum(O_{s,c,cw,t} - P_{s,c,cw,t})^2}{n}} \quad \text{Equation 3.3}$$

where $O_{s,c,cw,t}$ and $P_{s,c,cw,t}$ are the measured and estimated fraction survival (between 0 and 1) for species s, chemical c, exposure concentration C_w, and time t, respectively, and *n* the number of times the fraction survival was measured during the experiment. Second, the typical RMSE was determined by simply averaging the $RMSE_{Cw}$ values:

$$RMSE = \frac{\sum RMSE_{Cw}}{m} \quad \text{Equation 3.4}$$

where m denotes the number of experiments. The RMSE summarizes both random error and systematic bias [77].

3.3 Results

Overall, the estimated time-varying survival deviated from the measured survival dynamics for crustaceans and fish exposed to eight oil constituents. In general, the maximum effect of the oil constituents on the survival of several crustaceans and fish estimated with the model was reached within four days (Figures 3.1 and 3.2). Right after the onset of exposure, the model overestimated the lethal effect of pyrene and fluorene on *H. azteca* and pyrene and fluoranthene on *D. magna* (Figures 3.1a, b, d and e). The model also overestimated the lethal effect of fluoranthene on *H. azteca*, *Chironomus tentans* and *Diporeia* spp. during the first days of exposure (Figures 3.1c, f and g). Furthermore, we found that the estimated BBs of fluoranthene reached a steady state earlier than the measured BBs for *H. azteca* and *C. tentans* (Figure B1). For *Diporeia* spp. the BBs were overestimated during the first days of exposure days and underestimated at the last day of exposure (day 28).

The model underestimated the maximum mortality for most crustaceans except for *D. magna* exposed to fluoranthene (Figure 3.1e) and *Diporeia* spp. exposed to 250 µg/L fluoranthene (Figure 3.1g). Figures 3.1b and 3.1d show minor differences between estimated and measured survival for *H. azteca* and *D. magna* exposed to 698 µg/L fluorene and 70 µg/L pyrene, respectively. For fish, the model underestimated the mortality except for *Pimephales promelas* exposed to trimethylbenzene (Figure 3.2a) and to 6050 µg/L naphthalene (Figure 3.2b). The average uncertainty in the modelled effects on survival, expressed as the RMSE, was 0.25 with a minimum and maximum $RMSE_{Cw}$ of 0.04 and 0.67, respectively (Table 3.3). More specifically, the $RMSE_{Cw}$ ranged from 0.04 to 0.67 for crustaceans and from 0.07 to 0.55 for fish.

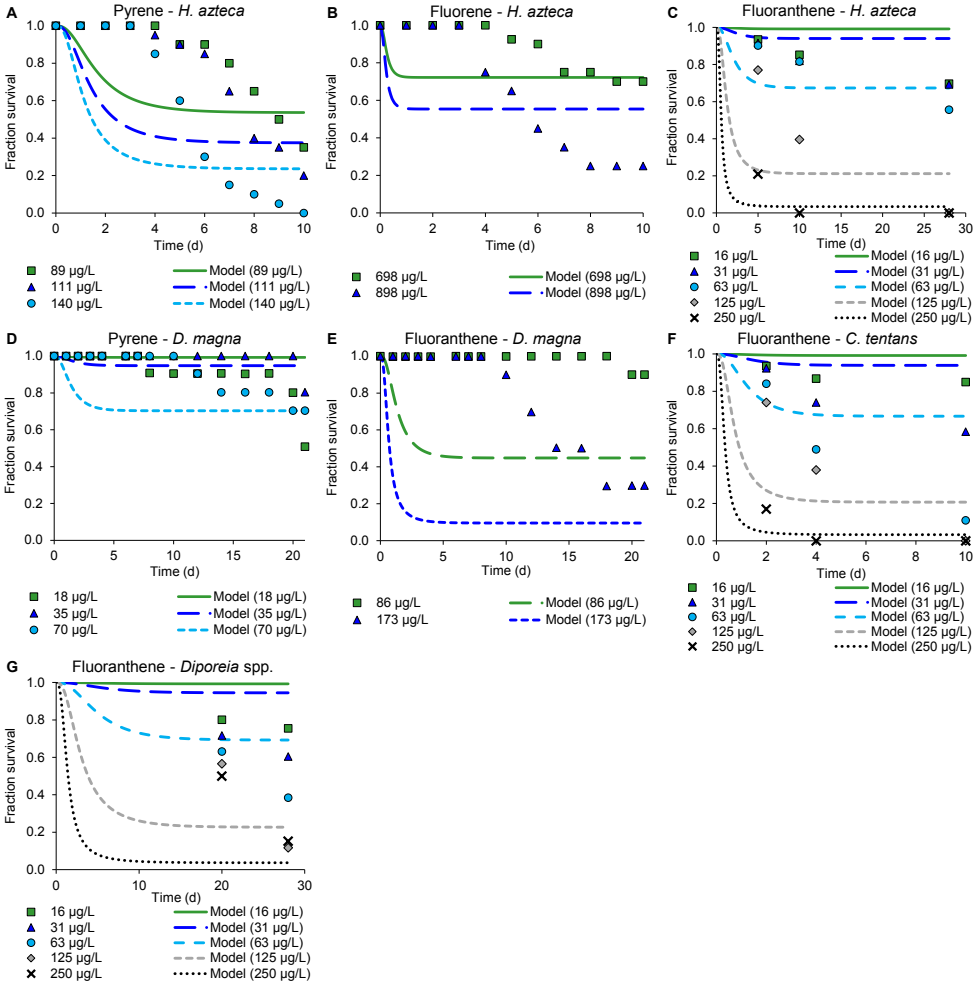


Figure 3.1 Fraction survival measured experimentally (dots) and estimated with Eqns. 3.1 and 3.2 (lines) for the crustaceans *Hyalella azteca* (A, B, C), *Daphnia magna* (D, E), *Chironomus tentans* (F) and *Diporeia* spp. (G) exposed to different concentrations of oil constituents.

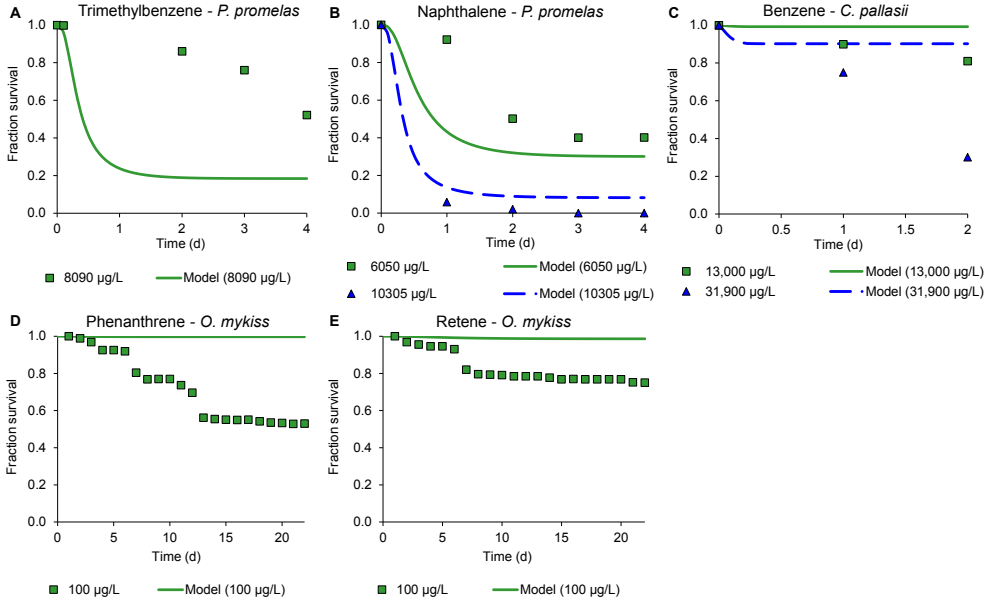


Figure 3.2 Fraction survival measured experimentally (dots) and estimated with Eqn. 3.1 and Eqn. 3.2 (lines) for the fish *Pimephales promelas* (A, B), *Clupea pallasii* (C) and *Oncorhynchus mykiss* (D, E) exposed to different concentrations of oil constituents.

Table 3.3 The number of data points (n) and root-mean-square-errors (RMSE) of the fraction survival of different aquatic organisms exposed to different oil constituents

Chemical	C_w (µg/L)	Species Latin name	Species common name	n	$RMSE_{cw}$	Ref.
Fluoranthene	16	*Chironomus tentans*	Midge	4	0.10	[a]
Fluoranthene	31	*Chironomus tentans*	Midge	4	0.21	[a]
Fluoranthene	63	*Chironomus tentans*	Midge	4	0.30	[a]
Fluoranthene	125	*Chironomus tentans*	Midge	4	0.27	[a]
Fluoranthene	250	*Chironomus tentans*	Midge	4	0.07	[a]
Pyrene	18	*Daphnia magna*	Water flea	15	0.15	[b]
Pyrene	35	*Daphnia magna*	Water flea	15	0.04	[b]
Pyrene	70	*Daphnia magna*	Water flea	15	0.20	[b]
Fluoranthene	86	*Daphnia magna*	Water flea	15	0.49	[b]
Fluoranthene	173	*Daphnia magna*	Water flea	15	0.67	[b]
Fluoranthene	16	*Diporeia* spp.	Amphipod	3	0.18	[a]
Fluoranthene	31	*Diporeia* spp.	Amphipod	3	0.24	[a]
Fluoranthene	63	*Diporeia* spp.	Amphipod	3	0.18	[a]
Fluoranthene	125	*Diporeia* spp.	Amphipod	3	0.21	[a]
Fluoranthene	250	*Diporeia* spp.	Amphipod	3	0.28	[a]
Fluoranthene	16	*Hyalella azteca*	Amphipod	4	0.17	[a]
Fluoranthene	31	*Hyalella azteca*	Amphipod	4	0.14	[a]
Fluoranthene	63	*Hyalella azteca*	Amphipod	4	0.14	[a]
Fluoranthene	125	*Hyalella azteca*	Amphipod	4	0.30	[a]
Fluoranthene	250	*Hyalella azteca*	Amphipod	4	0.09	[a]
Fluorene	698 [g]	*Hyalella azteca*	Amphipod	11	0.18	[c]
Fluorene	898 [g]	*Hyalella azteca*	Amphipod	11	0.30	[c]
Pyrene	89 [g]	*Hyalella azteca*	Amphipod	11	0.27	[c]
Pyrene	111 [g]	*Hyalella azteca*	Amphipod	11	0.36	[c]
Pyrene	140 [g]	*Hyalella azteca*	Amphipod	11	0.38	[c]
Benzene	13000	*Clupea pallasii*	Pacific herring	3	0.12	[d]
Benzene	31900	*Clupea pallasii*	Pacific herring	3	0.36	[d]
Phenanthrene	100	*Oncorhynchus mykiss*	Rainbow trout	15	0.40	[e]
Retene	100	*Oncorhynchus mykiss*	Rainbow trout	15	0.22	[e]
Naphthalene	6050 [g]	*Pimephales promelas*	Fathead minnow	5	0.24	[f]
Naphthalene	10305 [g]	*Pimephales promelas*	Fathead minnow	5	0.07	[f]
Trimethylbenzene	8090 [g]	*Pimephales promelas*	Fathead minnow	5	0.55	[f]
$RMSE_{model}$					**0.25**	

[a] [27], [b] [29], [c] [98], [d] [114], [e] [118], [f] [97], [g] The measured exposure concentration.

3.4 Discussion

In general, our study showed that the generic and dynamic OMEGA model, based on the Critical Body Burdens concept, overestimated the mortality right after the onset of exposure and underestimated the maximum mortality for crustaceans and fish exposed to oil constituents. The CBB approach thus failed to predict the dynamic effects of chemicals with a baseline toxicity (narcosis) on the survival of organisms. Below, we discuss potential reasons for the deviations found.

3.4.1 Model deviations

The geometric mean of measured LBBs (66 mmol/kg lipid) was in the range of the LBBs estimated by using QSARs for fish exposed to 124 narcotic chemicals, i.e. 40-160 mmol/kg lipid [5, 120, 121]. Additionally, the geometric mean LBBs determined for oil constituents (64 mmol/kg lipid) and narcotic chemicals excluding oil constituents (75 mmol/kg lipid) were significantly similar (p-value > 0.05; Table B3). The performance of the model improved slightly from a RMSE of 0.25 ($RMSE_{Cw}$: 0.04-0.67) to 0.23 ($RMSE_{Cw}$: 0.02-0.56) when optimizing the mean LBB from 66 to 89 mmol/kg lipid, because the reduced differences between measured and estimated mortality right after the onset of exposure outweigh the increased deviations at a maximum mortality.

Additionally, a sensitivity analysis was performed to evaluate the influence of the LBB on the model fit. Overall, a factor 2 lower LBB did not improve the average model performance (RMSE: 0.34 and $RMSE_{Cw}$: 0.02-0.84). A factor 2 higher LBB resulted in a similar average RMSE of 0.25 compared to no change in LBB, but the $RMSE_{Cw}$ range improved slightly to 0.01-0.48. In particular, the difference between survival estimates and measurements reduced by 46-78% for *D. magna* exposed to fluoranthene and 67% for *P. promelas* exposed to trimethylbenzene (Table B5, Figures B2 and B3). Nevertheless, the model still overestimated the survival fraction in the first days of chemical exposure. Additionally, species-specific and chemical-specific measured LBBs were reported for *H. azteca*, *C. tentans* and *Diporeia* spp. exposure to fluoranthene: namely, 71, 19 and 85 mmol/kg lipid, respectively [27]. The relatively low LBB for *C. tentans* indicated higher species sensitivity to fluoranthene. Yet, when estimating the survival by using the species-specific LBB instead of the narcotic LBB, the $RMSE_{Cw}$ for C. tentans exposed to different fluoranthene concentrations increased from 0.07-0.30 to 0.08-0.45. Concluding, the LBB influences the model performance for few species exposed to specific aromatic hydrocarbons, but the sensitivity analyses indicated no general pattern for all exposure concentrations. For example, the model fit right after the onset of exposure remained erratic.

The average slope (i.e. 1/ß) of 3.0 for internal concentrations was similar to a previously reported slope of 3.1 (min-max: 0.6-4.8) of the external concentration-response curves of crustaceans exposed to chemicals with a narcotic TMoA [122]. The average slope of 4.2 for four oil constituents was higher than the slope of 2.7 for narcotics excluding oil constituents (Table B3). The best possible model fit, that is a RMSE of 0.22 ($RMSE_{Cw}$: 0.03-0.50) instead of 0.25, was obtained by reducing the slope from 3.0 to 1.1, thereby suggesting a very high inter-individual variation in LBBs. A sensitivity analysis showed a change in average RMSE from 0.25 to 0.27 ($RMSE_{Cw}$: 0.00-0.75) and 0.22 ($RMSE_{Cw}$: 0.04-0.54) using a factor 2 lower and higher slope, respectively (Table B5, Figure B2). Overall, the factor 2 higher slope slightly reduced the difference between estimates and measurements, in particular for *Diporeia* spp. exposed to fluoranthene (11-46% reduction). In line with the LBB, the slope influences the model performance for

few species, but indicated no general pattern for all exposure concentrations.

In four survival experiments a nominal exposure concentration $C_{w,c}$ was reported [27, 29, 114, 118]. Although test solutions were changed daily of every other day to achieve the initial concentration specified, sorption and volatilisation could have contributed to a reduced water concentration. We evaluated if exposure concentration and time could be explanatory variables for the degree of deviation between the estimated and measured survival. A factor underestimation or overestimation per data point, calculated using $P_{s,c,cw,t}/O_{s,c,cw,t,}$ was related to the corresponding time t or exposure concentration $C_{w,c}$ by using linear regression. Over all species and oil constituents, the relative deviation showed a significant positive trend in relation to $C_{w,c}$ and time (*p*-values are 0.04 and <0.01, respectively). Yet, these trends for $C_{w,c}$ and time explained only 1.7% and 3.1%, respectively, of the variation in the estimated/measured ratios (Figure B3).

The estimated fraction survival reached a steady state situation earlier than observed in the experiments. This could partly be explained by overestimated BBs in the first exposure days, as shown for *H. azteca, C. tentans* and *Diporeia* spp. exposed to fluoranthene (Figure B1). We evaluated the performance of the kinetic part of the model by calculating the RMSE using log-transformed measured and estimated BBs in Eqns. 3.3 and 3.4. The model overestimated the BBs of fluoranthene in *H. azteca* by a factor of 1.4 to 1.7 (factor = $10^{\wedge RMSE}$) and in *C. tentans* by a factor of 1.3 to 2.0 (Table B6). For *Diporeia* spp. the BBs were overestimated for the first exposure days and underestimated at the last day, resulting in an overall overestimation by a factor of 1.3 to 2.5.

Overestimation of the BBs, and thus mortality, right after the onset of exposure may be partly explained by a possible underestimation of the weight or lipid fraction of the organisms. Except for the lipid weight of *H. azteca*, *C. tentans* and *Diporeia* spp. exposed to fluoranthene, we used values obtained from other experimental studies. An underestimated weight would lead to overestimated absorption and elimination rates, causing the maximum estimated mortality to be reached more quickly compared to the measured mortality. We performed a sensitivity analysis to evaluate the influence of the weight and lipid fraction on the model fit. We set both variables on no change and an order of magnitude decrease and increase (Table B7). Overall, a factor 10 decrease and increase in wet weight had a small impact on the relative error ($RMSE_{Cw}$: 0.05-0.69 and 0.04-0.63 respectively) compared to no change in wet weight ($RMSE_{Cw}$: 0.04-0.67). Except for *H. azteca* exposed to pyrene, *P. promelas* to naphthalene (6050 µg/L) and *C. tentans* to fluoranthene (125 µg/L) the RMSE reduced by 22 to 40% using a factor 10 increase in wet weight (Table B7). An order of magnitude change in lipid fraction resulted on average in lower model performance as the RMSE increased from 0.25 to 0.28 ($RMSE_{Cw}$: 0.03-0.76) and 0.26 ($RMSE_{Cw}$: 0.01-0.73) using a factor 10 lower and higher lipid fraction, respectively. The model fit improved with 41 to 68% using a factor 10 higher lipid weight for some individual cases: *D. magna* exposed to 173 µg/L fluoranthene, *Diporeia* spp. to 250 µg/L

fluoranthene, *H. azteca* to fluorine and *P. promelas* to trimethylbenzene (Table B7). A factor 10 deviation in wet weight is however expected to be more likely than a similar high deviation in lipid weight. Eventually, one order of magnitude change in the input variables wet weight and lipid fraction did not produce a general improvement of the model performance (Figures B3a, B3b and B4).

The exclusion of biotransformation rates ($k_{3,out}$) of oil constituents in crustaceans may also contribute to the overestimation of mortality. Our model included biotransformation as an additional elimination route for the parent compound and excluded the possible body burdens of products formed by biotransformation. An underestimated elimination rate due to exclusion of biotransformation would therefore lead to overestimated BBs and mortality. Only a metabolic transformation rate of 1.15 ± 0.1 day^{-1} and 0.06 pmol $\cdot min^{-1} \cdot g^{-1}$ has previously been reported for *H. azteca* and *P. platyceros* exposure to fluoranthene and benzo(a) pyrene, respectively [87, 115]. However, after including a biotransformation rate of 1.15 d^{-1} in the model, the differences between the estimated and measured time-varying survival decreased for *D. magna*, yet increased for *H. azteca* (see Figure B5). Furthermore, this particular biotransformation rate was not included in the model estimations, because in the survival experiment with *H. azteca* the body burdens were expressed as total fluoranthene equivalent residues, that is the total internal concentration of parent and metabolite compounds [27].

Narcosis was the suggested TMoA of the parent and metabolite compounds for fluoranthene, justifying body burden addition [27]. Metabolites could also exhibit a more specific toxicity than narcosis, for instance some metabolites of phenanthrene can cause toxic effects by a nonnarcotic and nonphototoxic mode of action in juvenile fish [123]. Some parent PAHs are also known to cause specific (chronic) effects, such as cardiotoxicity [124] and dioxin-like aryl hydrocarbon receptor-mediated effects [125]. For fish, the QSARs used to predict biotransformation rates do not provide predictions for the formation of metabolites, some of which may be at least as toxic as the parent compound [81]. Nevertheless, in the current study differences between the modelled and measured survival for retene (dioxin-like TMoA) are comparable with the differences of the other oil constituents with an expected narcotic TMoA.

In a toxicity study with a light and heavy oil type it was suggested that the toxicity of heavy oil is higher due to a TMoA other than narcosis: physical soiling. Very heavy oil constituents may contribute to physical soiling of the organisms depending on the amount of oil present in the sediment [48]. In the current study, the molecular mass of the oil constituents ranged between 78 g/mol for benzene and 234 g/mol for retene. Although the performance of our model was similar for the light and heavier chemicals, it should be taken into account that physical effects might also contribute to a reduced survival of organisms.

3.4.2 Model assumptions

The body burden was immediately linked to survival in our model because we assumed a steady state to occur rapidly for chemicals with baseline toxicity [97]. However, especially for *H. azteca* and *D. magna* exposed to pyrene, fluoranthene and fluorene no effect was observed in the first four to eight days of the experiment, respectively, resulting in a large deviation between the measured and estimated mortality. If the time-varying body burdens cannot explain the time course of survival, alternative approaches could be used. For example, it could be assumed that the body burden leads to damage which in turn leads to mortality [27, 97]. Damage would then be used as a dose metric to simulate delayed effects in the toxicodynamic part of the model [126].

In accordance with previous studies, the LBB of chemicals with a narcotic TMoA was assumed to be independent of exposure-related parameters such as time and concentration [121, 127]. In various studies, this concept of a constant LBB (e.g. in the Critical Body Residue Model) has been tested by measuring LBBs and the exposure duration until mortality occurred (time-to-death) of aquatic species exposed to organic chemicals. Depending on the method used, the LBB varied or remained constant over time. For example, within one experimental treatment (e.g. one exposure aquarium) the variation in organism sensitivity lead to an increase in LBB with increasing exposure duration for *P. promelas* exposed to naphthalene and 1,2,4-trichlorobenzene [128] and *H. azteca* exposed to three PAHs [26, 98]. In contrast, comparing a mean LBB and exposure duration over different treatments resulted in a decreased or a constant LBB with time for two fish, a crab and amphipod species exposed to biocides, chlorobenzenes and PAHs [26, 128]. Despite these contrasting outcomes, these findings indicate that temporal variation in the effects of oil constituents on the survival of aquatic species may not only be due to time-varying body burdens, but also to changes in LBB with increasing exposure duration [26].

In the current study the model was based on the individual tolerance (IT) hypothesis. An alternative hypothesis is stochastic death (SD), which assumes that all individuals have an equal chance of dying and the probability to die increases when exceeding the LBB [110]. The individual sensitivities of crustaceans and fish in the experiments were unknown because they were not measured; therefore both model hypotheses could have been applicable. To evaluate the performance of the model when assuming SD, the fraction survival was estimated by calculating the probability that an individual survives until the next day given a certain chemical concentration. The fraction survival on day n was subsequently calculated by multiplying the survival probabilities of all preceding days (see Appendix B for equations). A comparison of the measured and estimated effects for crustaceans and fish mainly showed an overestimated mortality when using a model with SD assumptions (Figure B6), which underlined that neither one of the model hypotheses is most valid for toxicodynamic modelling. This is in accordance with experimental and modelling studies that

estimated the survival of *Gammarus pulex* in propiconazole exposure [110] and the time to stupefaction in zebra fish (*Brachydanio rerio*) exposed to benzocaine and lethality in mosquitofish (*Gambusia holbrooki*) exposed to sodium chloride [129].

3.4.3 Implications and recommendations

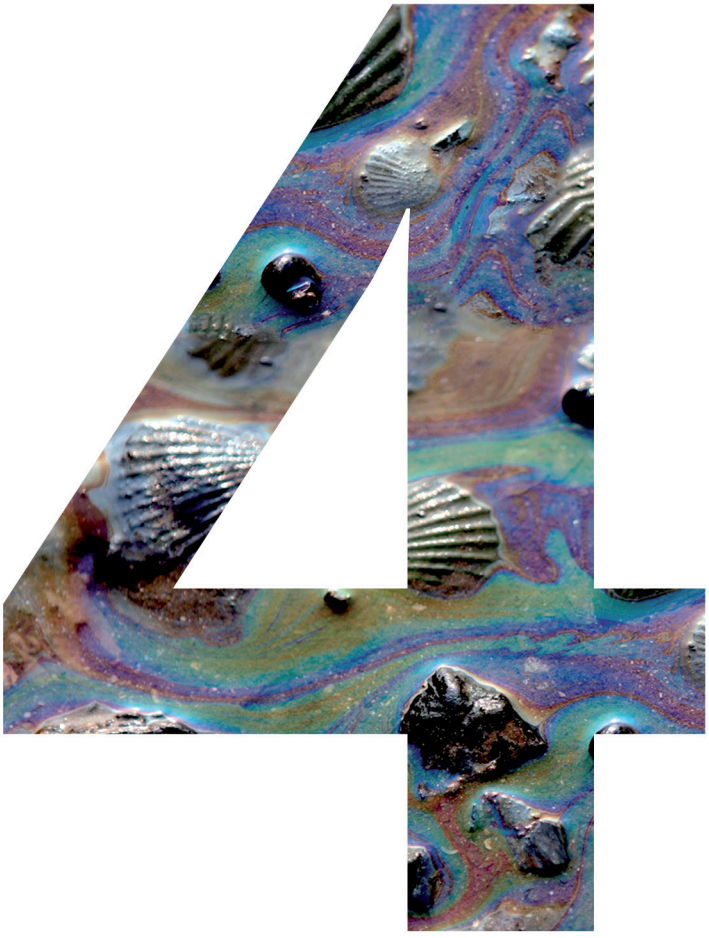
A visually comparison of our results to the results of the DEBtox model [29, 97], a TKTD model, showed that the DEBtox model fitted better to the measured survival data than the OMEGA model for *D. magna* exposed to pyrene and fluoranthene and *P. promelas* exposed to trimethylbenzene. For *P. promelas* exposed to naphthalene, performance was comparable between both models. Compared to OMEGA, the DEBtox model includes more information on energy fluxes in organisms, such as the volume-specific costs for structure and fraction of reserve flux to maturation [130]. Yet, experimental observations needed as input for DEBtox can be missing for species and chemicals as most toxicity experiments are not designed with a DEB-based analysis in mind [131].

We assumed the exposure concentration to be constant over time which is in accordance with the survival experiments in which the test solutions were changed daily or every other day [27, 29, 97, 98]. Contrastingly, in field situations concentrations of oil can decrease rapidly due to processes such as physical dilution [132]. Exposure conditions after open ocean spills are therefore expected to be of short duration (e.g. hours), which is in the range where our model overestimated the mortality. In theory, the model can be used for fluctuating exposure concentrations; yet constant exposure concentrations already yielded deviations that require additional research.

In conclusion, the estimated time-varying survival generally deviated from the measured survival dynamics for crustaceans and fish exposed to eight oil constituents. The average uncertainty in the generic OMEGA model, expressed as the RMSE, was 0.25 (min-max: 0.04-0.67) on a scale between 0 and 1. Thus, the model based on the CBB approach failed to adequately predict the lethal effects of chemicals with a baseline toxicity (narcosis). Possible explanations for the deviations between model estimates and observations may include uncertainties in model parameters as well as incorrect assumptions regarding the absence of biotransformation products, the constant LBB and the steady-state of aromatic hydrocarbon concentrations in organisms. Model performance might be improved by including a delay between accumulation and effect, e.g. by addition of a damage factor like is done in the Damage Assessment Model [126], a time-varying LBB instead of a constant LBB or toxic effects induced by biotransformation products. In short, a more complex model approach than the generic approach used in this study is needed to predict toxicity dynamics of narcotic chemicals.

3.5 Acknowledgements

We thank Isabel O'Connor for her help with the model performance statistics, and Aaron Redman, Tone Karin Frost and Roman Ashauer for their suggestions that helped improving this manuscript. We thank the Norwegian Research Council (NRC) for support through the PETROMAKS program (BIP project #ES468602). The SYMBIOSES project is a cooperation of 15 research partners, financed by the NRC, BP Exploration Operating Company Limited, ConocoPhillips Skandinavia, ExxonMobil Upstream Research Company, Eni Norway, Shell Technology Norway, Statoil Petroleum and Total E&P Norway.



Chapter 4

Crude oil affecting the biomass of the marine copepod *Calanus finmarchicus*: comparing a simple and complex population model

Lisette de Hoop
Ole Jacob Broch
A. Jan Hendriks
Frederik de Laender

Published in Marine Environmental Research 119: 197-206.

Abstract

In the current study differences were evaluated between a complex 3D multistage population model (SINMOD) and a simpler consumer-resource population model for estimating the effects of crude oil on the marine copepod *Calanus finmarchicus*. The SINTEF OSCAR model was used to simulate hypothetical oil spills in the Lofoten area in 1995, 1997, and 2001. Both population models simulated a negligible effect of crude oil on the *Calanus*' biomass when assuming low species sensitivity. The simple model estimated a larger effect on the biomass (up to a 100% decline) compared to the complex model (maximum decline of 60 to 80%) at high species sensitivity to crude oil. These differences may be related to the inclusion of copepod advection in the complex model. Our study showed that if little data is available to parameterize a model, or if computational resources are scarce, the simple model could be used for risk screening. Nevertheless, the possibility of including a dilution factor for time-varying biomass should be examined to improve the estimations of the simple model. The complex model should be used for a more in depth risk analysis, as it includes physical processes such as the drift of organisms and differentiation between developmental stages.

4.1 Introduction

The copepod *Calanus finmarchicus* plays an important role in the structure, stability, and function of marine ecosystems on the Norwegian continental shelf [133]. *C. finmarchicus* dominates the marine mesozooplankton communities south of the Polar Front in terms of production and standing biomass [134]. Many fish species, such as the Atlantic mackerel (*Scomber scombrus*), Atlantic herring (*Clupea harengus*) and larvae of the Atlantic cod (*Gadus morhua*), feed on the plankton fields with *C. finmarchicus* [135-137]. Given their importance, the impact of crude oil on *C. finmarchicus* should be assessed to support environmental decisions to protect subarctic and Arctic marine ecosystems.

In order to improve the understanding of the effects and quantify the risks of oil exposure, numerical models are useful. Models that have been developed to assess the spatial and temporal distribution of plankton can also be used to assess the effects of chemicals. For example, SINMOD, a three-dimensional hydrodynamic-ecological model system including a structured population model for *C. finmarchicus*, has been used to assess the potential impacts of contaminated waste water discharges on the reproduction and biomass of *C. finmarchicus* [20]. Simpler, one-dimensional population models, which require less information about the physical habitat (e.g. hydrodynamics), have also been used to estimate the effects of organic chemicals and metal concentrations on single-species or consumer-resource dynamics of phytoplankton and zooplankton species [105, 108, 138].

Predictions with complex population models can be used to evaluate the impact of physical processes, such as the transport and weathering of oil, on ecosystem effects. However, numerical models require parameterized data which is generally only available for a limited number of species and chemicals. The more complex the model, the more parameterized data is needed. Extrapolation of data across species or between chemicals causes uncertainties in the input-data used in the models for untested species and chemicals, and produces unknown uncertainties in the output-data from the models. A simpler population model may already give an indication of the chemical impact on population dynamics in, for instance, worst-case situations. Based on an extensive review of population modelling in ecological risk assessment (ERA) of chemicals, Forbes et al. recommended to assess the performance of different types of population models – from simple to complex – in terms of providing outputs relevant for ERA [30]. Such a comparison should especially be performed for the subarctic and Arctic marine regions, where the food chains are relatively short compared to other regions. The ERA of chemical exposure to a key species as *C. finmarchicus* is important, because adverse effects on key species could have a large impact on the entire ecosystem, especially in ecosystems with a short food chain. However, until now, it has not been tested how estimates of the effects of crude oil on *C. finmarchicus* biomass in the subarctic compare between population models with different levels of complexity.

The aim of our study was to evaluate the difference between the multistage SINMOD population model and a simpler consumer-resource population model (Rosenzweig-MacArthur) for estimating the effects of crude oil on the marine copepod *C. finmarchicus*. Both population models were coupled to the OMEGA bioaccumulation model, which included concentration-response functions to estimate crude oil effects on the copepods' survival and reproduction (SINMOD) or on copepod survival only (Rosenzweig-MacArthur model). We simulated relative change in biomass over time with the complex and simple population models with and without exposure to crude oil in a spill simulated by an oil spill fate and exposure model. Additionally, we evaluated the influence of two toxicological parameters: the lethal body burden (LBB; the internal concentration which causes lethality for 50% of the tested organisms) and the slope of the concentration-response curves on the model output.

4.2 Methods

4.2.1. Model specification

Oil fate and exposure model. Time-varying oil concentrations of dissolved hydrocarbons were obtained from the three-dimensional SINTEF OSCAR (Oil Spill Contingency And Response) model, which simulates the fate, distribution and composition of crude oil in the marine environment after a discharge [139, 140]. Driving forces are wind and ocean currents. The model uses up to 25 pseudo-components to represent a light crude oil. Behaviour of each pseudo-component is determined by a set of chemical and toxicological parameters determining the solubility, volatility and aquatic toxicity [74]. The oil concentration fields for all pseudo-components calculated in OSCAR were exported as input to the 3D population model (§ *Complex population model*). For the simple population model, the maximum concentrations of each pseudo-component over the top 50 meters of the water column were calculated (hourly values) for a grid cell close to the release point and used in the bioaccumulation calculations (§ *Bioaccumulation model*).

Bioaccumulation model. The OSCAR model was coupled with the OMEGA bioaccumulation model in order to estimate a time-varying body burden (BB) per pseudo-component in *C. finmarchicus* (Figure 4.1). The BB is estimated based on the uptake of the dissolved chemical from the water and elimination rate constants from the copepod. These rate constants are quantified as a function of the octanol-water partition coefficient (K_{ow}) of the chemical and the organism's wet weight, lipid content and trophic level [6]. The BBs were numerically calculated, as they were assumed to depend on the internal concentration in the previous time step. Accumulation in phytoplankton was not taken into account. A detailed description of the model algorithms and parameter values can be found in Hendriks et al. [6] and Chapter 2, Appendix A1. The K_{ow} and molecular mass of the 25 pseudo-components are available in Table C1 in the Appendix.

The BBs of all pseudo-components (p) were summed to determine the total body burden (TBB; mmol/kg lipid) of crude oil in *C. finmarchicus* per time step (t) according to;

$$TBB_t = \sum BB_{p,t} \qquad \text{Equation 4.1}$$

In previous studies, it was confirmed that the effects of chemical mixtures with a baseline toxicity can be explained by this sum, which corresponds to the concentration addition model of mixture toxicity [14, 112, 116, 141]. We assumed that all oil constituents exhibit baseline toxicity, i.e. a narcotic toxic mode of action (TMoA), based on the classification of chemicals by Verhaar et al. [117, 142, 143].

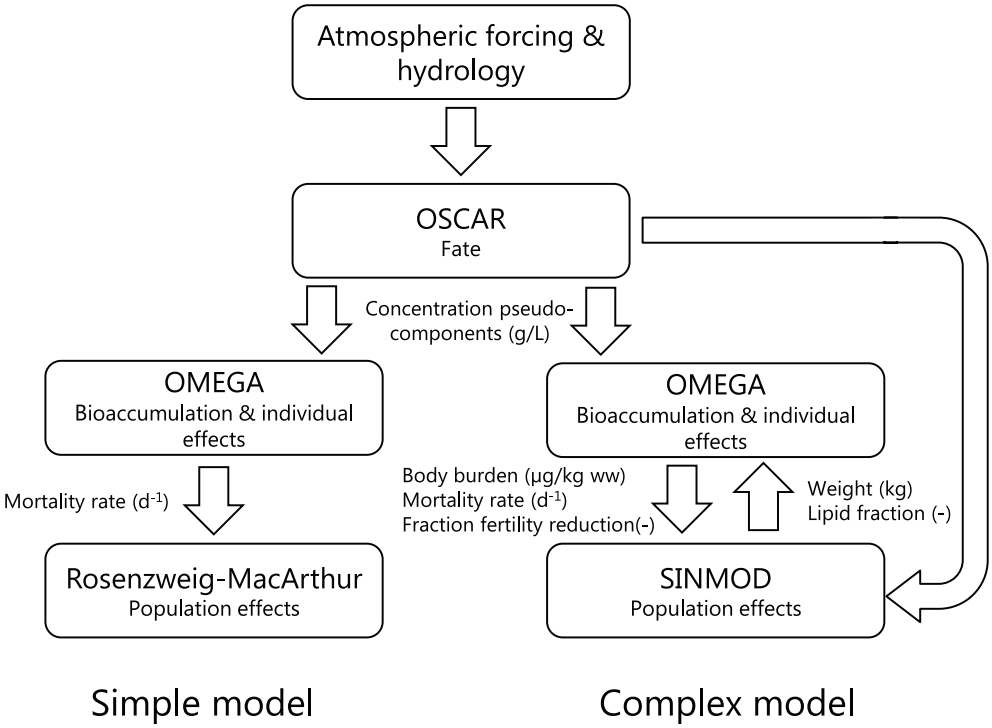


Figure 4.1 Model components with their exported variables for the simple and complex models, with the Rosenzweig-MacArthur and SINMOD models delivering the output.

Simple population model. Using the simple population model the effects of crude oil on the survival of *C. finmarchicus* were calculated based on the OSCAR output (Figure 4.1) and per time step t using the estimated TBB_t and single-species toxicity data as represented by concentration-response function [39, 144];

$$k_{m,oil,t} = T_{exp}^{-1} \ln(1 + (\frac{TBB_t}{LBB})^{slope}) \qquad \text{Equation 4.2}$$

where $k_{m,oil,t}$ is the mortality rate (d^{-1}), LBB is the lethal body burden (mmol/kg lipid), the slope is of concentration-effect curves and T_{exp} the experimental test duration (days), respectively. The model assumed an stochastic death distribution meaning that eventually all individuals in a population are affected at a body burden that leads to mortality, because individuals are assumed to have a similar sensitivity to crude oil [97]. An overview of the LBBs and slopes of concentration-response curves for three different toxicity settings are available in Table 4.1. Additional details on the origin of these values are presented in Table C2. The LBBs are comparable to the range of LBBs derived for other aquatic organisms by McGrath and Di Toro [145].

Table 4.1 Parameter values based on oil toxicity data for crustaceans, for use in modelling the impact of oil components on zooplankton

Parameter settings	Parameter [a]	Value	Unit
Average: average ecotoxicological parameter values are given, assuming medium species sensitivity, yet taking into account differences in the duration of the toxicity test.	LBB	83.76	mmol/kg lipid
	slope	5.36	/
	q_{ls}	1.14	/
	T_{exp}	10	d
Worst-case: the oil spill has maximal ecotoxicological effects. The ecotoxicological parameter values that lead to the highest effect on the copepod population are chosen (high species sensitivity).	LBB	12.30	mmol/kg lipid
	slope	11.11	/
	q_{ls}	1.06	/
	T_{exp}	10	d
Best-case: the oil spill has minimal ecotoxicological effects. The ecotoxicological parameter values that imply the lowest effect on the copepod population are chosen (low species sensitivity).	LBB	200	mmol/kg lipid
	slope	2.65	/
	q_{ls}	1.38	/
	T_{exp}	28	d

[a] Parameters in Eqns. 4.2 and 4.5, where LBB is the lethal body burden, the slope is of concentration-effect curves, q_{ls} the lethal-sublethal ratio and T_{exp} the experimental test duration.

The mortality rates (i.e. $k_{m,oil,t}$) were included in the Rosenzweig-MacArthur food chain model to examine crude oil effects on the population dynamics of *C. finmarchicus* [15, 138]. In this model, the natural growth of *C. finmarchicus* was related to the availability of food (i.e. phytoplankton). The population densities of phytoplankton (N_1; gC·m^{-2}) and copepods (N_2; gC·m^{-2}) were described as a function of time t with parameters linked to the body mass of an individual organism [15];

$$\frac{dN_1}{dt} = r_1 \times (1 - \frac{N_1}{K_1}) \times N_1 - \max(k_{n,2}) \times \frac{N_1^\beta}{N_1^\beta + N_{50,1}^\beta} \times N_2 \qquad \text{Equation 4.3}$$

$$\frac{dN_2}{dt} = (p_{an,2} \times p_{pa,2} \times \max(k_{n,2}) \times \frac{N_1^\beta}{N_1^\beta + N_{50,1}^\beta} - k_{m,2}) \times N_2 \qquad \text{Equation 4.4}$$

where $k_{m,2}$ is the mortality rate of copepods and $\max(k_{n,2})$ is the maximum consumption rate constant of copepods. The mortality rate $k_{m,2}$ is equal to the maximum value of two mortality rates: 1) the background mortality $k_{m,b}$ or 2) the mortality due to oil exposure $k_{m,oil,t}$ for *C. finmarchicus* [144]. We assumed no effects of crude oil on phytoplankton abundance and copepod reproduction in this simple model due to the transient nature of the exposure and the relatively rapid growth of the algae during spring [146]. A description and calculation or typical value of the variables and parameters used in the Rosenzweig-MacArthur model are available in Table C3.

4.2.2 Complex population model

The estimated TBB_t values in *C. finmarchicus* were used in Eqn. 4.2 to determine the mortality rate ($k_{m,oil,t}$) due to crude oil exposure. The effects of crude oil on the reproduction of C. finmarchicus (Eqn. 4.5; dimensionless) were additionally calculated based on the OSCAR output (Figure 4.1) and per time step according to [144];

$$Fraction\ fertility\ reduction_t = \frac{1}{1+(q_{ls}\times\frac{TBB_t}{LBB})^{slope}} \qquad \text{Equation 4.5}$$

where q_{ls} is the lethal-sublethal ratio, which is the ratio between the concentrations of a chemical resulting in a lethal effect and a sublethal effect (e.g. for 50% of the organisms), respectively. In the current study, this is equal to the ratio between external concentrations that have an effect on the survival and reproduction of organisms, as no internal concentrations were available. An overview of the qls values for three different parameter settings is available in Table 4.1. Additional details on the origin of these values are presented in Table C2.

The mortality rate and fraction fertility reduction were integrated into the 3D physical-biological SINMOD model that simulates the distribution, concentrations and interactions of the lower trophic levels of the planktonic ecosystem (Figure 4.1). The model includes state variables for nutrients, dissolved organic carbon, bacteria, detritus, phytoplankton, ciliates and the mesozooplankton species *C. finmarchicus* and *Calanus glacialis* [147, 148]. The model structure and parameters used in the biological model are described in Wassmann et al. [149]. In addition, a stage structured population model for *C. finmarchicus* was integrated in SINMOD in order to simulate different life cycle stages. The egg and nauplii development consists of nine compartments and the copepodites' weight development of a total of 29 compartments, representing all physiological developmental levels CI to CVI [148]. Each of the developmental stages are represented by 2D concentration fields with a fixed vertical distribution used for advection in 3D. Body burdens for each of the 25 oil pseudo-components used, for each of the 29 levels, are included in the model. The concentration field *C=C(w,t)* of a weight class of *C. finmarchicus* at time t is described by the differential equation;

$$\frac{\partial C}{\partial t} + g_w\frac{\partial C}{\partial w} + \sum_{b=1}^{25}(a_b - e_b)\frac{\partial C}{\partial B_b} + Adv(C) + Diff(C) = Birth - Mortality \qquad \text{Equation 4.6}$$

where g_w is the growth rate, w is the weight (in µgC) and Adv and Diff are the advection and diffusion operators [149, 150]. The rates a_b and e_b are the absorption and elimination rates, respectively, of oil pseudo-component b for the weight w, calculated from the OMEGA model, while B_b are the body burdens [6]. The present version of the *Calanus* model uses a fixed relationship between the weight and developmental stage [151], and the growth rate is determined as a function of temperature and food availability [152]. The *Calanus* model does not simulate lipid content dynamically, so in order to use the OMEGA model, a fixed

relationship between lipid content (WL; µg lipid·ind^{-1}) and developmental level (lev) has been assumed: $WL(lev)=0.119\ \exp(0.2319 lev)$, $lev=1,\ldots,28$; $WL(29)=65$. The exponential relationship between level and weight [148, 153] justifies the exponential model for absolute individual total lipid content, but it should be noted that in reality the relative lipid content and composition may vary substantially [154]. Base mortality rates for the copepodite stages was assumed to lie between 0.055 and 0.025 for the CI to CV stages, and 0.013 and 0.039 d^{-1} for the adult females (CVI). See [152] for further details.

The SINMOD setup used here has a 4000 m horizontal resolution and covers the Norwegian coast. The model is nested from a 20,000 m resolution model covering the Nordic and Arctic seas. Atmospheric forcing is applied using ERA-Interim data from the ECMWF (European Centre for Medium-Range Weather Forecasts) [155], and freshwater runoff from land has been supplied by NVE (Norwegian Water Resources and Energy Directorate).

The main differences between the simple and complex population models are given in Table 4.2.

Table 4.2 Key differences between the simple (Rosenzweig-MacArthur) and complex (SINMOD) population models

Model elements	Simple model	Complex model
C. finmarchicus life stages (including wet weight and lipid fraction)	1	29
Species or species groups	2 (copepods, diatoms)	5 (bacteria, phytoplankton, ciliates, *C. finmarchicus*, *C. glacialis*)
Feed types for *C. Finmarchicus*	Diatoms	Diatoms and ciliates
C. finmarchicus population mobility (in space and time)	Closed, excluding transport processes	Open, including transport processes (e.g. advection, mixing, migration, sinking)
Type of crude oil exposure effects on *C. Finmarchicus*	Reduction of fraction survival	Reduction of fraction survival and fraction fertility
Calculations which include *C. finmarchicus* mortality due to crude oil	Copepod biomass	Copepod biomass and total body burden (feedback loop)
Parameterization type	All allometric relations, except diatom wet weight and intrinsic growth rate copepods	Continuous weight development rates calculated from food availability and
Computational needs	Computationally undemanding	Computationally demanding. Need for High Performance Computing facilities

4.2.3 Oil spill scenario

A sea surface oil spill was modelled from a platform situated at Nordland VI point 2, south-west of the Lofoten-Vesterålen islands (67.216044° N, 11.33558° E; Figure 4.2), which appears to be one of the most prospective areas for petroleum resources on the Norwegian shelf [156]. Starting from March 19 there was a hypothetical top-side release of 4500 tons of light crude oil per day over a period of 50 days. This represents a massive, unmitigated spill that is approximately half the volume and duration of the Deepwater Horizon oil spill [157]. No response, such as the use of dispersants or mechanical clean up, was assumed. The discharge period coincides with the presence of *C. finmarchicus* in the top layer of the water column during the spring [158, 159]. Furthermore, an experimental study has shown increased movement of *C. finmarchicus* towards the light during exposure to crude oil constituents, causing a larger proportion of copepods to remain in the surface waters [160]. Time-varying concentrations for 25 pseudo-components were determined for a model grid cell (4000m x 4000 m x 3 m) and for a region of 21 x 21 grid cells (84 x 84 km) at the shelf of the Lofoten archipelago (Figure 4.2). Overall, the light crude oil consisted mainly of benzenes, naphthalenes and C8- and C9-saturates. See Table C4 for the crude oil composition at the start and end of the hypothetical oil spills in the years 1995, 1997 and 2001.

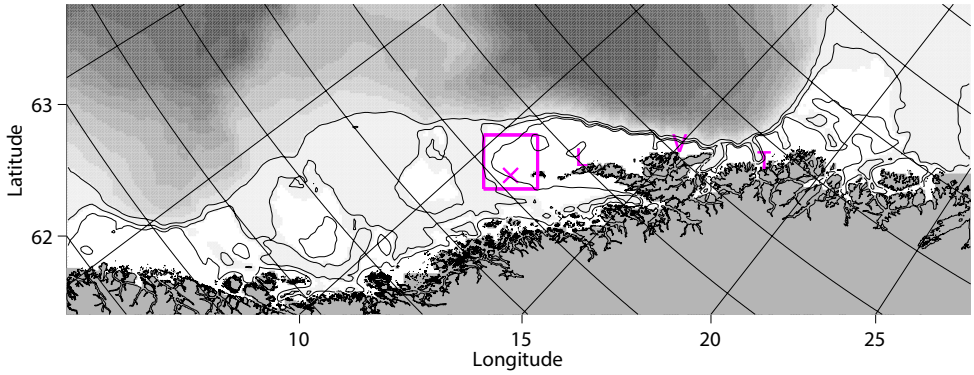


Figure 4.2 SINMOD model domain of 4 km horizontal resolution used in the present study. The approximate position of the oil release point and point for extracting *Calanus* time series (magenta X) is indicated. The magenta square indicates the larger sub region of the model domain (21 x 21 grid cells) used for extracting spatially averaged *Calanus* time series. The black curves are 200, 300 and 500 m isobaths. The magenta letters indicate the Lofoten (L) and Vesterålen (V) archipelagos and the city of Tromsø (T).

We simulated the *C. finmarchicus*' biomass (gC $\cdot m^{-2}$) with the simple and complex population model over a minimum time span of 100 days, including a 50 day oil discharge period, for three different years with contrasting hydrodynamic conditions and spatially different *C. finmarchicus* populations. On March 19, average biomass of all *Calanus* life stages ranged between 0.5 and 0.9 gC $\cdot m^2$ in the years 1995, 1997 and 2001. The oil spill scenarios for the three different years were obtained from SINTEF and have previously been used to test the performance of SYMBIOSES: an integrated modelling framework for ecosystem-based impact assessment and management [22].

4.2.4 Comparing the simple and complex model

First, we simulated a time-varying absolute biomass with the simple and complex population model by using the three oil spill scenarios and a baseline scenario (no crude oil exposure).

For comparison between the simple one-dimensional and complex three-dimensional model, the biomass estimated by using the 3D SINMOD model was depth averaged to determine the *Calanus* biomass (gC$\cdot m^{-2}$) for one model grid cell and a region of 21 x 21 grid cells (84 x 84 km).

Second, for each model the relative change in biomass (%) at time t between an oil spill and baseline scenario was calculated according to;

$$Relative\ biomass\ change_t = \frac{biomass_{t,oil} - biomass_{t,base}}{biomass_{t,base}} \times 100 \qquad \text{Equation 4.7}$$

where biomasst,oil and $biomass_{t,base}$ are the biomass of *C. finmarchicus* (gC $\cdot m^{-2}$) estimated with and without exposure to crude oil concentrations.

For comparison of body burdens, the TBB at time *t* was averaged over all *C. finmarchicus* developmental compartments in SINMOD as follows;

$$TBB_t = \frac{\sum_n TBB_n w_n Ab_n}{\sum_n w_n Ab_n} \qquad \text{Equation 4.8}$$

Here, TBB_n denotes the TBB_t (mmol/kg lipid) of *C. finmarchicus* level n, w_n is the individual weight of level n, and Ab_n is the abundance of level n. The sums are taken over all developmental levels.

4.3 Results

4.3.1 Absolute biomass and relative difference from baseline

Biomass simulations of *C. finmarchicus* near the release point of crude oil showed differences between the complex multistage SINMOD model (Figure 4.3) and the simpler consumer-resource Rosenzweig-MacArthur population model (Figure 4.4). The highest average estimated copepod biomass is a factor 7.4, 3.6, and 4.2 higher in 1995, 1997, and 2001 respectively, when using the complex model compared to the simple model for the baseline scenario. The density at day 0 (i.e. 19 March) is similar for each year when using the simple model, but differs between years for the complex model. After simulating a decline in the biomass of *C. finmarchicus*, both models simulated an increase again when assuming no oil spill. The biomass increased after 40 days in the complex model and after 130 days in the simple model.

For all three years, both models estimated a negligible effect of crude oil on *C. finmarchicus*' biomass assuming low sensitivity of the zooplankton species. Only the simple model showed a short decrease in biomass in 1997. The two models estimated a different impact of crude oil on the copepod biomass when using average and worst-case oil toxicity parameter values (causing average and highest effects) (Figures 4.3 and 4.4). In contrast to the rapid 100% biomass decline in the simple model, a maximum decline of 60-80% in biomass was estimated in the complex model when assuming high species sensitivity to crude oil (Figures 4.3b and 4.4b). When assuming average sensitivity on *C. finmarchicus* crude oil had a minor impact on biomass in the complex model compared to the simple model, which simulated a 100% biomass decline in the 1997 scenario. In the 1995 and 2001 scenarios the simple model simulated a fluctuating biomass with a maximum decrease of 70% and 65%, respectively. The maximum biomass was reached 54 and 29 days before the baseline biomass reached a maximum in 1995 and 2001, respectively. For comparison to Figure 4.4b, Figure 4.3b only shows the relative difference of biomass from the baseline between -100% and 20%. However, the relative difference is higher than 20% when assuming average sensitivity of *C. finmarchicus* in the 1995 and 2001 scenarios and assuming low sensitivity in the 1997 scenario (maximum of 1543%, 773% and 170%, respectively).

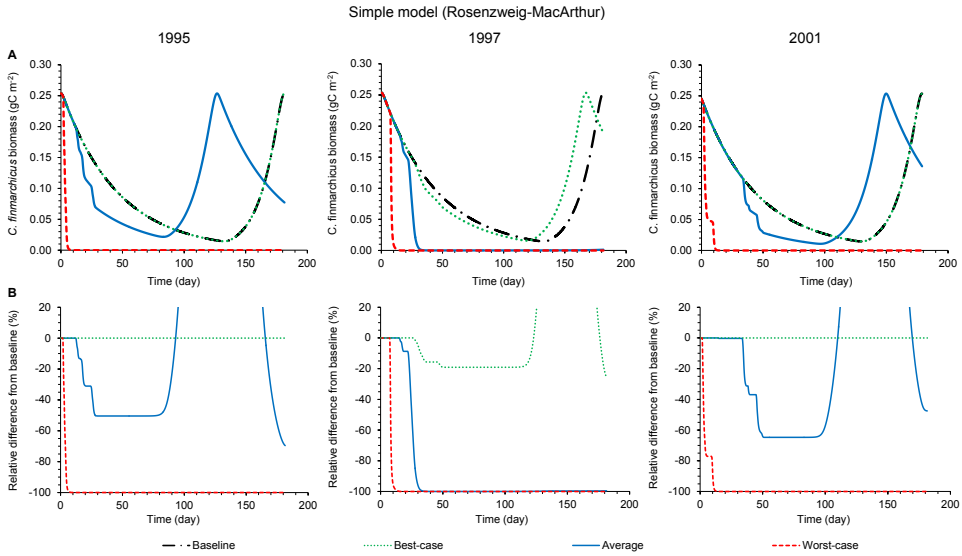


Figure 4.3 Time series of the A) average *C. finmarchicus* biomass (gC·m^{-2}) and B) relative difference (%) in *C. finmarchicus* biomass between the baseline and three oil different oil toxicity parameter settings at a point close to the "Nordland VI – point 2" release point of crude oil in the years 1995, 1997 and 2001 for the Rosenzweig-MacArthur consumer-resource model. The best-case, average and worst-case ecotoxicological parameter values cause low, average and high effects of crude oil on the organism (Table 4.1), and the baseline represents simulation data without oil exposure. The lines for the baseline, average and best-case parameter settings are overlapping. The relative biomass difference from the baseline is not plotted above 20%.

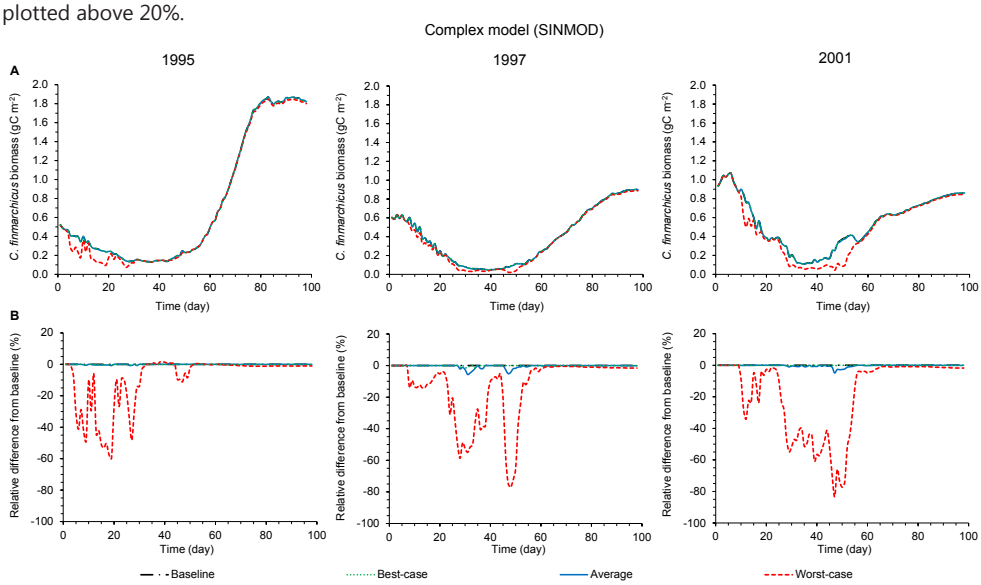


Figure 4.4 Time series of the A) average *C. finmarchicus* biomass (gC·m^{-2}) and B) relative difference (%) in *C. finmarchicus* biomass between the baseline and three oil toxicity parameter settings at a grid cell close to the "Nordland VI – point 2" release point of crude oil in the years 1995, 1997 and 2001 for the SINMOD population model. The baseline and oil toxicity parameter settings are similar to Fig. 4.3, but note the differences in time scales. The lines for the baseline and best-case ecotoxicological parameter values are overlapping.

In addition to the simulated biomass for a grid cell (4 km x 4 km) near the release point of crude oil (Figure 4.4), the biomass was simulated for a larger region (21 x 21 grid cells, i.e. 84 km x 84 km) in the 2001 scenario in SINMOD (Figure 4.5). There is a negligible effect of crude oil on the simulated *C. finmarchicus*' biomass in a large region compared to the effects on the copepods in a smaller area near the oil release point (Figure 4.4). After 50 days of exposure to crude oil 77.3% (0.29 gC $\cdot m^{-2}$) and 12.6% (0.04 gC $\cdot m^{-2}$) of the *C. finmarchicus* biomass has been lost near the release point and in the larger region compared to the baseline simulation when assuming high species sensitivity, respectively.

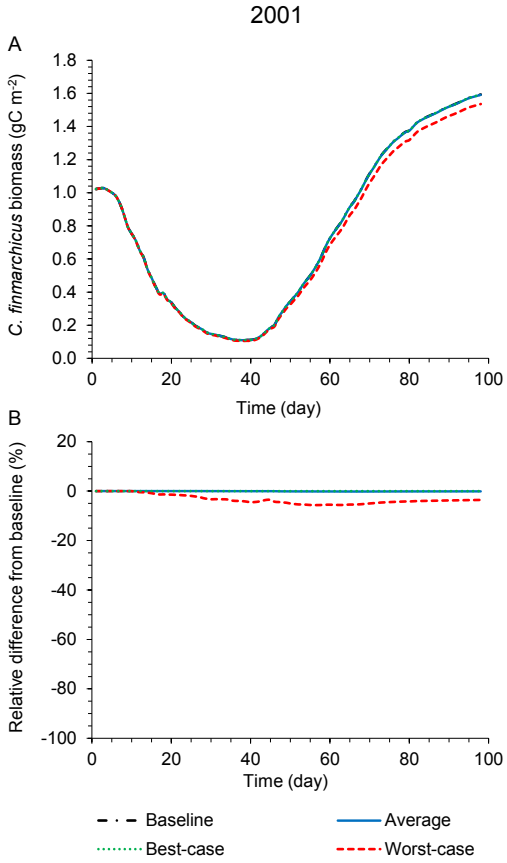


Figure 4.5 Time series of the A) average *C. finmarchicus* biomass (gC$\cdot m^{-2}$) and B) relative difference (%) in *C. finmarchicus* biomass between the baseline and three oil toxicity parameter settings at a region (21 x 21 grid cells) near the "Nordland VI – point 2" release point of crude oil in the year 2001 for the SINMOD population model. The baseline and oil toxicity parameter settings are similar to Fig. 4.3. The lines for the baseline, best-case and average ecotoxicological parameter values are overlapping.

4.3.2 Total body burdens

Figure 4.6 shows that the maximum TBBs estimated by using the complex multistage SINMOD model are a factor 3.2, 3.6, and 2.6 lower than the ones estimated by using the simpler Rosenzweig-MacArthur consumer-resource population model for the years 1995, 1997 and 2001, respectively. In contrast to the simple model, the complex model estimated different TBBs when using the worst-case ecotoxicological parameter values compared to the best-case and average parameter values. The crude oil concentrations in the water column near the oil release point (i.e. the sum of 25 pseudo-components) are highest in the 1997 scenario.

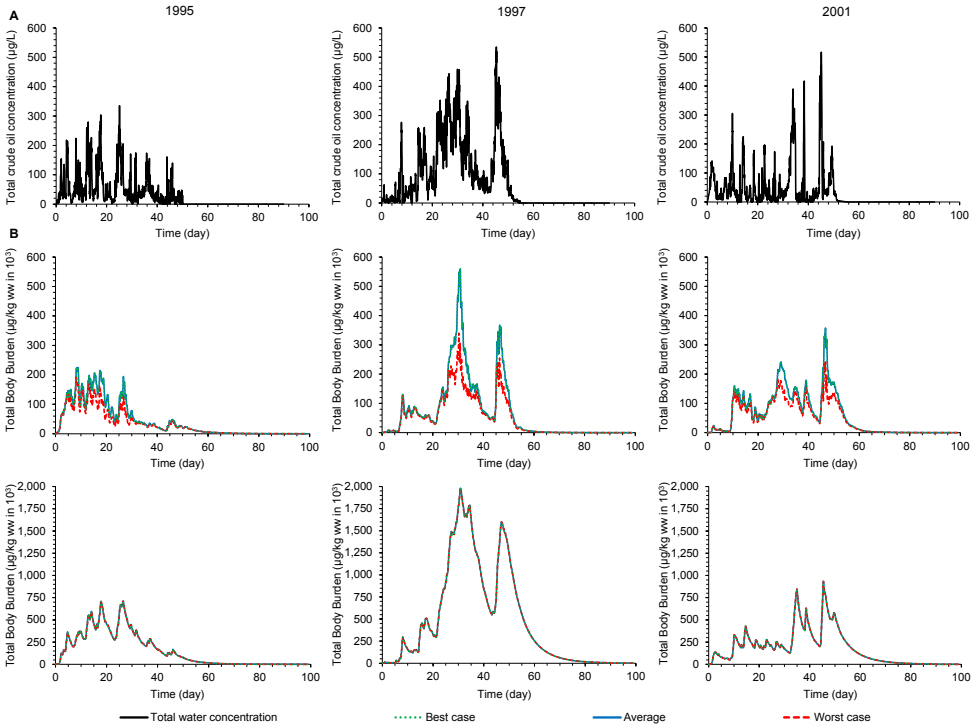


Figure 4.6 A) Concentration of crude oil in water (µg/L), and total body burdens (µg/kg wet weight) of crude oil in the copepod *C. finmarchicus* estimated by using the OMEGA bioaccumulation model in B) the SINMOD population model and C) the Rosenzweig-MacArthur consumer-resource population model at a point close to the "Nordland VI – pkt 2" release point in the years 1995, 1997, and 2001. The best, average and worst-case ecotoxicological parameter values cause low, average and high effects of crude oil on the organisms (Table 4.1). Note the different scaling of the y-axes in B and C. The lines for the average and best-case parameter settings are overlapping for both models.

4.4 Discussion

4.4.1 Baseline biomass simulations for C. finmarchicus

The *C. finmarchicus* biomass estimated by using the complex multistage SINMOD model and the simpler consumer-resource Rosenzweig-MacArthur model is in the same order of magnitude as the measured biomass in the Norwegian Sea ranging from 0.05 to 6.3 $gC \cdot m^{-2}$ between March and June of the year 1997 [161]. The simple model estimated a reduction of the copepod biomass due to the assumption that the food availability decreased with increased predator biomass. For the complex model, the decline in biomass with a minimum after about 40 days of simulation (early May, in all years) reflects the dynamics of the population structure, and does not represent a decrease in total abundance. Initially there are mainly adult copepods, while later the biomass consists predominantly of nauplii in greater abundances, but with significantly lower individual biomass. The biomass increases again as the nauplii develop into copepods. The biomass fluctuations simulated by using the complex model are in agreement with the biomass pattern measured in Disko Bay near Greenland [162]. *C. finmarchicus* is adapted to an environment with a regular yearly spring bloom, as occurs in the Norwegian Sea. The organisms spawn in April and May during or just after the bloom peak and develop from stage I copepodites to stage V copepodites by June or July. These copepodites over-winter in deeper water and develop into sexually mature adults in January. Between January and March a lot of energy is invested in the development of ovaries in females, resulting in large decreases in body mass [163].

The difference in biomass fluctuations and hence differences in time scales between the simple and complex model may be partly related to assumptions made in the simple model. Allometric relations were used to parameterize the simple population model (Rosenzweig-MacArthur model) based on species weight and allometric exponents, except for the diatom weight (food of *C. finmarchicus*) and the intrinsic growth rate (r_1) of diatoms. The used average r_1 of 0.6 d^{-1} for cultured diatoms from the Barents Sea was similar to the allometrically determined r_1 (0.68 d^{-1}) based on the diatom weight and average production coefficient [15].

4.4.2 C. finmarchicus biomass exposed to crude oil

In the simple model the maximum baseline biomass was reached after simulating 180 days of *C. finmarchicus* fluctuations. In the 1995 and 2001 scenarios the maximum biomass was reached 29 to 54 days earlier when assuming average species sensitivity to crude oil. This might be related to the relation between the copepod and its single food source (diatoms) in the simple model. As the copepod biomass decreased rapidly during the simulated oil spill, the diatom biomass was able to increase due to a lack of grazers. After the oil spill stopped, the copepods were able to recover rapidly due to a surplus of food. In the field the diatoms might be effected by oil as well, therefore the simple model could overestimate

the fast recovery period of *C. finmarchicus*. Nevertheless, *C. finmarchicus* has multiple feed sources in the field with a feeding selectivity which is influenced by the quantity and quality of the available food [164].

Body burdens. The estimated total body burdens of *C. finmarchicus* were different between the simple and complex population model. In both models the same bioaccumulation equations were used to estimate TBBs from the same exposure concentrations near the oil release point, but the estimates from the complex model were approximately a factor of 3 smaller than those from the simple model. This difference is partially related to the inclusion of the advection of copepods in space and time in the complex model and partially to the short exposure time of the copepods to the highest concentrations (a few hours) [20]. In contrast to the simpler model, the complex model included the copepod mortality per time step due to oil exposure in the calculation of the copepod abundance and TBBs in a grid cell. During the simulated oil spill the copepods with a high TBB died or moved to another grid cell in the model, causing a reduction in the overall TBB. The exposure of crude oil would not be higher to fewer copepods per se, as clean copepods could enter the grid cell in the model. Eventually, the advection of copepods contributed to a dilution of internal crude oil concentrations in time and space of the complex model.

Furthermore, the estimated TBBs in the complex model were lower when assuming high species sensitivity (using the worst-case ecotoxicological parameter values) compared to the TBBs when assuming average and low species sensitivity (using the average and best-case parameter values). The worst-case parameter values caused a higher mortality rate, resulting in more organisms that die out and thus a slightly lower overall TBB in a grid cell compared to the other two ecotoxicological parameter settings.

The advection of copepods by ocean currents probably plays an important role in maintaining individual populations and in maintaining connections between populations of *C. finmarchicus* [165]. The advection of copepods could also be accounted for in the simpler model. For example, by including a dilution rate affecting the copepod biomass as has been done by Gentleman et al. [166], or by including an immigration term [167]. The simple model would be equal to a 'closed population', which has been defined as a population contained in an isolated water mass by Gentleman et al. [166]. The complex model would be equal to an 'open population', which is affected by transport processes, such as advection, mixing, sinking and migrations.

The impact of crude oil on the biomass of *C. finmarchicus* was additionally evaluated using similar TBBs in the simple and complex models. The TBBs (mmol/kg lipid weight) that were estimated by the simple model were divided by a factor 3.2 (1995), 3.6 (1997) and 2.6 (2001) in order to achieve approximately the same TBBs as those estimated by the complex model. Using these adjusted TBBs in the simple model resulted in 1) a 100% decrease of the *C. finmarchicus*'

biomass compared to the baseline when assuming highest species sensitivity to crude oil, 2) a maximum decrease in biomass of 20% when assuming average species sensitivity in the 1997 scenario, and 3) no effect of crude oil on the biomass when assuming low species sensitivity (Figure C1). The complex and simple models therefore simulated similar effects on the copepod biomass when using similar TBBs. Except, in the simple model the biomass reduced a 100% compared to the baseline biomass whereas the biomass in the complex model maximally reduced by 80% and revived again after 50 days.

In conclusion, the simple model simulated a larger effect on copepod biomass than did the complex model, mainly due to the higher TBBs and thus higher mortality rates estimated by the former. At similar TBBs, the models simulated overall similar effects of crude oil on time-varying *C. finmarchicus* biomass, except the estimated effects in the simple model were larger compared to those in the complex model.

Mortality rates. In a previous study, the mortality rate formula (Eqn. 4.2) has been used to estimate relative differences of phytoplankton and zooplankton biomass between a control situation and treatments with copper [144]. From these simulated relative differences, highly accurate no-observed-effect-concentrations (NOECs) were derived on a population and ecosystem level. It was suggested that the quality of the toxicity data that are used will influence the estimations, but that this is also the case with conventional extrapolation techniques [144].

In the current study, the simple and complex models used the same ecotoxicological parameter values and could therefore not contribute to differences between the predictions by two models. There are some assumptions which could have caused uncertainties in the mortality rate estimations in both models. The single-species oil toxicity data were only obtained for freshwater crustaceans, but similar have been derived for other species [145, 168]. Furthermore, the minimum and maximum values of the obtained toxicity data were used for the best-case and worst-case ecotoxicological parameter settings. The worst-case LBB of 12.3 mmol/kg lipid weight was obtained from an experimental study using an insect instead of a crustacean. This was the absolute worst-case value found among empirical LBB data for aquatic species. Large differences between minima and maxima could cause large differences of crude oil effects on the biomass between the best-case and worst-case ecotoxicological parameter settings. Figure C2 illustrates the relationship between estimated mortality rates and TBBs when using Eqn. 4.2. Average parameter values were therefore used to obviate a comparison between most extreme situations.

The simple and complex models included the effect of crude oil on the survival of *C. finmarchicus*, and the complex model also included effects on the reproduction of *C. finmarchicus*. Nevertheless, due to advection of copepods and the temporal development of *C. finmarchicus* the impact of crude oil on the fertility of *Calanus*

females will be simulated by the complex model at a location different than the oil release point. Therefore the effect of crude oil on the fraction fertility reduction will not be depicted in the biomass simulations near the release point (one grid cell) in SINMOD.

Small versus large simulation domain. For the 2001 scenario the effect of crude oil on the biomass of *C. finmarchicus* was simulated for one grid cell (4 km x 4 km) and a larger area (84 x 84 km) near the oil release point. The results showed that the effect of crude oil on the copepod biomass was negligible in the larger area compared to the point estimates, implying that the size of the simulation domain is important for the effects seen on *C. finmarchicus*. This means that although the impact on the copepod biomass could be high near an oil release point, as simulated by both the simple and complex model, caution is required when extrapolating the biomass effects from a small area to a population in a larger region.

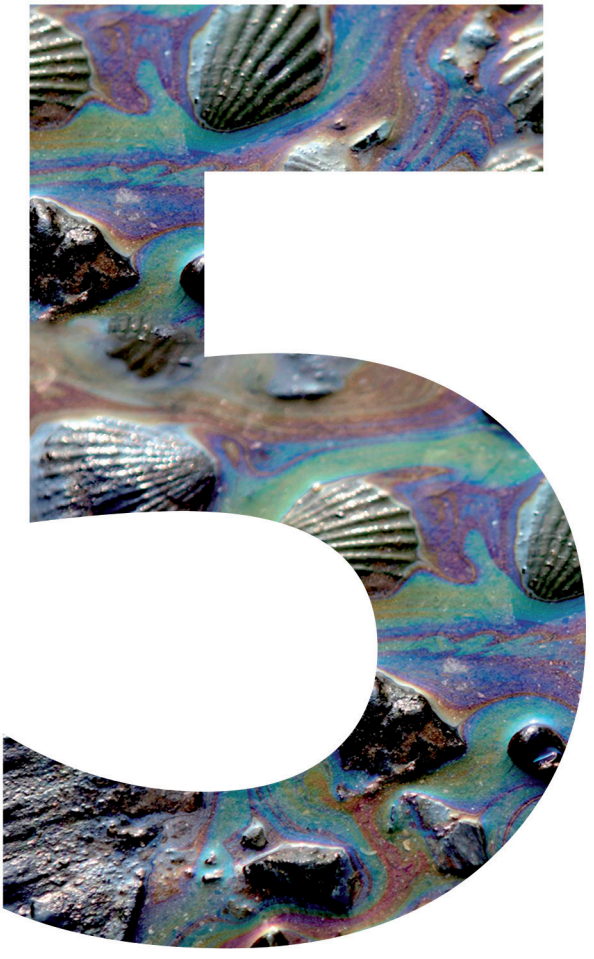
4.4.3 Conclusion and recommendations

The complex multistage SINMOD population model and the simpler consumer-resource Rosenzweig-MacArthur population model simulated a negligible effect of crude oil on the biomass of *C. finmarchicus* assuming low species sensitivity to crude oil (best-case ecotoxicological parameter values). The simple model simulated a 100% biomass decline compared to the baseline biomass when assuming average species sensitivity in the 1997 scenario and high species sensitivity in all scenarios (1995, 1997 and 2001), whereas the complex model simulated a negligible biomass decline and a 60 to 80% biomass decline, respectively. The TBBs estimated by the simple model were on average a factor of three higher than those from the complex model, causing higher estimated mortality rates. This difference in TBBs was probably caused by the inclusion of copepod advection in the complex model, resulting in the dilution of TBBs. When using similar TBBs, both models simulated overall similar effects of crude oil on time-varying *C. finmarchicus*' biomass, except the effects in the simple model were still higher compared to those in the complex model.

In conclusion, our study showed that if few data are available to parameterize a model, or if computational resources are scarce, the simple model could be used for risk screening. Yet, when assuming high species sensitivity the simple model simulated extreme effects of crude oil on *C. finmarchicus*. The coupled bioaccumulation and 3D complex multistage SINMOD model should be used for a more in depth risk analysis, as it includes physical processes such as the drift of organisms. To improve the estimations of the simple model, the possibility of including a dilution factor for time-varying biomass should be examined. In the future, oil spill models that address the impact on biota could be validated if field data measured before and after an oil spill become available.

4.5 Acknowledgements

The current paper is inspired through the SYMBIOSES project, as the coupling of the fate (SINTEF OSCAR), bioaccumulation (OMEGA) and complex multistage population model (SINMOD) was performed in this project. SYMBIOSES is a cooperation of 15 research partners, financed by the Norwegian Research Council (NRC), BP Exploration Operating Company Limited, ConocoPhillips Skandinavia, ExxonMobilUpstream Research Company, Eni Norway, Shell Technology Norway, Statoil Petroleum and Total E&P Norway. The NRC is acknowledged for support through the DEMO 2000 program (project #235150). The authors thank the Steering Committee of the SYMBIOSES project for their contribution to the paper.



Chapter 5
Modelling toxic stress by atrazine in a marine consumer-resource system

Lisette de Hoop
Marleen de Troch
A. Jan Hendriks
Frederik de Laender

Published in Environmental Toxicology and Chemistry 32 (5):1088-1095

Abstract

The present study combines short-term experiments with food chain modelling to explore long-term effects of the herbicide atrazine on consumer-resource dynamics in a marine intertidal ecosystem. The short-term (28 days) lab experiments indicated that the intrinsic rate of increase (r) and carrying capacity (K) of the marine diatom *Seminavis robusta* decreased with increasing atrazine exposure. This decrease did not show the concave shape expected from the diatoms' lifetime productivity for the nonexposed diatoms, and single-species toxicity data from the literature but instead was described best by a linear model. These experimentally observed atrazine-induced decreases of r and K were used to parameterize a Rosenzweig-MacArthur model representing a simple food chain including the tested diatom and its grazer, the harpacticoid copepod *Delavalia palustris* var. *palustris*. Stable oscillation zoobenthos-phytobenthos systems were produced at diatom exposures of 0, 100 and 150 µg/L atrazine. An atrazine concentration of 150 µg/L contributed to a 15% increase of the oscillation periods of both diatoms and copepods and a 52% reduction of diatom oscillation amplitudes compared to the control situation. Although the amplitudes of copepods only increased 7% at 150 µg/L atrazine, the maximum and minimum copepod density at that concentration were reduced with 61% and 63%, respectively. The effects of atrazine on periodicity and amplitudes were robust to 20% changes in the food chain model parameters that represented allometric relationships. Our simulations suggest food chain mediated indirect effects on zoobenthos populations, indicating a reduced diatom and copepod availability throughout the year.

5.1 Introduction

Population dynamics have been studied frequently to understand why and how population abundances change in space and time [169]. Stress from abiotic factors, such as extreme temperatures, floods and contaminants can influence the population dynamics drastically [31, 170, 171]. First signs of environmental stress can be observed in field surveys as a declining population size [172]. Field surveys are a direct method of establishing an effect of stressors on population dynamics. Yet, it may be difficult to pinpoint the causes for the observed dynamics in a continuously changing environment [172]. To overcome the difficulties of field studies, population dynamics may be studied in experimental microcosms with controlled conditions [172]. In addition to single-species laboratory tests, microcosms allow for toxic stress responses to be examined on interactions between a few species. Furthermore, these interactions are easier to monitor compared to multi-species field situations. Eventually, mechanisms by which toxicants influence the structure and function of populations at multiple trophic levels can be determined in microcosms [173].

Nevertheless, microcosm experiments have their limitations too. Only a minor fraction of the numerous naturally occurring populations can be studied experimentally because of difficulties of rearing them in laboratory conditions [174]. Mathematical models can be applied to overcome some of these limitations, improving the understanding and estimating of population dynamics [18]. Yet, for species with unknown sensitivities of growth rate-determining parameters this approach yields uncertain predictions. Ideally, models and empirical studies on the impact of a (toxic) stressor allow investigating effects on, e.g. ingestion, assimilation and reproduction rates, and thus on population parameters such as growth rate [175]. Models may then be used to explain how different types of dynamic behaviour, including oscillations, damping or stability, can occur and how different species life characteristics can give rise to similar dynamics [174].

So far, most models focus on either single-species or multi-species systems [18, 31, 176]. The consumer-resource dynamics of two interacting species under contaminant stress has hardly been studied. For example, Halbach et al. simulated oscillations of rotifers continuously fed with algae using a logistic model with a time lag. The model quantified adverse effects of the pesticide pentachlorophenol (PCP) on rotifers without additional assumptions about species interactions [33]. Furthermore, Fussmann et al. applied a mechanistic model with four differential equations to study dynamic behaviour qualitatively, producing cycles, equilibria and extinction in a rotifer-algae system with nitrogen limitation [32]. Functions of evolutionary trade-off between algal growth rates and predation defences refined the model to better fit empirical data [177]. In another study, the reproductive toxicity of PCP probably induced the observed density fluctuations of a rotifer consumer-resource system in a Lotka-Volterra model [16]. The intrinsic rate of increase r of populations was suggested to be representative of toxic effects on species interactions [16].

Most of the existing ecotoxicological food chain or food web models have typically been designed to represent specific species and contain parameters that are often difficult to measure in a laboratory setting [178]. The objective of the present study was to use a non-species-specific food chain model to explore consumer-resource dynamics in a marine ecosystem. The food chain model uses allometric scaling to infer population-level parameters that are difficult to measure experimentally for this ecosystem. Previous studies showed zoo-phytoplankton dynamics to be comparable between predictions of this model type and observations in field communities [179]. First, we performed short-term experiments to infer effects of the herbicide atrazine on the intrinsic rate of increase r and carrying capacity K of phytobenthos (the diatom *Seminavis robusta*) growth. Next, we used concentration-response models fitted to these data to parameterize the food chain model and explore how the dynamic behaviour of the marine zoo-phytobenthos system changes with increasing atrazine concentrations. Atrazine, highly soluble and persistent in water, interferes with the photosynthesis function of plants, including some algae, making it a suitable stressor for examination of indirect effects on the zoobenthos [180], here the copepod *Delavalia palustris* var. *palustris*. The assumed independence of the zoobenthos parameters from atrazine was checked by empirically testing if grazing rate or diatom (i.e. food) quality were significantly changed by atrazine. The sensitivity of model output to the uncertainty surrounding the allometric relationships was tested using a sensitivity analysis.

5.2 Materials and methods

5.2.1 Microcosm experiments

Laboratory conditions. Cultures of the epipelic diatom *Seminavis robusta* were grown in plastic culture flasks (surface = 175 cm²) with 200 ml autoclaved, artificial seawater (salinity 32 prepared with Instant Ocean® salt) and f2 medium as a nutrient source [181]. The cultures originated from strain 89A-S of the diatom culture collection of the Laboratory of Protistology and Aquatic Ecology, Ghent University, Belgium (http://www.pae.ugent.be/collection). Previous studies showed *S. robusta* to be successful as food source for several harpacticoid copepods in consumer-resource experiments [182]. Individuals of the benthic copepod *Delavalia palustris* var. *palustris* (family *Miraciidae*, further referred to as *D. palustris*) were collected from an intertidal area of the Westerschelde estuary in the Netherlands in November 2011. The harpacticoid copepods were kept in small Petri dishes (polystyrene, diameter = 5.2 cm) with artificial seawater without food (salinity 32, Instant Ocean® salt) for two days prior to the experiment. Diatoms cultures, copepods and all experimental units were kept at 15.5 ± 0.5 ˚C under a 12:12 hour light-dark regime.

Experimental design. To examine the effects of atrazine on the density and quality of diatoms, *S. robusta* was exposed to six atrazine concentrations (0, 1, 10, 20, 40, 100 µg/L) for four weeks. These atrazine concentrations were based on effect concentrations (EC_{50}) of atrazine on freshwater phytoplankton communities in a microcosm study [183]. All atrazine treatments were triplicated.

In a grazing experiment short-term effects of atrazine via phytobenthos on zoobenthos were examined. Diatom cells were labelled with the stable isotope ^{13}C to measure the uptake of cells grown under different atrazine concentrations by the copepod *D. palustris* (see labelling technique of De Troch et al.) [184]. Based on the experiment with *S. robusta* triplicated atrazine treatments (0, 20 and 100 µg/L) were used. Diatoms were exposed to atrazine solutions with labelled medium for five days. After replacing the solutions by artificial seawater (salinity 32), 20 copepods were added to each experimental unit for four days.

Both experiments were performed in glass Petri dishes (diameter = 8.8 cm). Each Petri dish received $2.9 \times 10^5 \pm 4.8 \times 10^4$ (mean ± SD) diatom cells per microcosm in artificial seawater with f2 medium. Experiments started by replacing the seawater with f2 medium by 20 ml of atrazine solution per microcosm. Atrazine solutions were based on a quantity of atrazine (400 mg atrazine in 1 L EtOH) diluted to the desired concentration using artificial seawater with f2 medium (salinity 32).

Analytic techniques and data treatment. In the first experiment diatom cell densities were counted under a Zeiss Axiovert 135 microscope (Zeiss Gruppe, Jena, Germany) in order to generate growth curves. To test if atrazine effects

on diatoms could propagate to copepods as changes in nutritional quality we measured diatom quality in two ways: the maximum quantum yield of photosynthetic activity (Fv/Fm) and total fatty acid (FA) concentrations. The Fv/Fm ratio was measured with Pulse-Amplitude-Modulation (PAM) fluorometry. The experiment was terminated by harvesting and subsequently centrifuging diatom cells for 5 minutes at 18°C and 2000 RCF (relative centrifugal force). The cells were stored in the freezer at -80°C until fatty acid (FA) analysis. The composition and total concentration of FA in the diatom cells were analysed according to the methods of De Troch et al. using a Hewlet Packard 6890N GC coupled to a mass spectrometer (HP 5973) [185]. The protocol is available in Text section D1 in Appendix D.

The uptake of diatom cells by copepods was determined by stable isotope analysis to test whether effects on diatoms could contribute to effects on copepods by changes in carbon assimilation. Prior to the grazing experiment, triplicate samples of diatoms and copepods from the field were stored at -20°C as a control for initial ^{13}C content. After four days of grazing, the survival rate was estimated in each microcosm and all live copepods were stored at -20°C. Diatom cells were harvested, centrifuged for five minutes at 18°C and 2000 RCF and stored at -80°C. Within two hours after defrosting, the copepods were thawed, washed five times in deionised water and transferred into tin capsules (8×5 mm, Elemental Microanalysis Limited) using a micro needle (to avoid label leakage). Per replicate also one droplet of highly concentrated diatom cells in artificial seawater was transferred into tin capsules using a micro pipette. All capsules were dried overnight at 60°C and finally pinch closed. For each replicate, $\delta^{13}C$ values of both diatoms and copepods and the copepod biomass (total carbon) were measured with a continuous flow isotope ratio mass spectrometer (type Europa Integra) at the UC Davis Stable Isotope Facility (University of California, USA).

Incorporation of ^{13}C is reflected as excess (above background) ^{13}C and is reported as (1) specific uptake ($\Delta\delta^{13}C = \delta^{13}C\text{sample} - \delta^{13}C\text{control}$ where $\delta^{13}C$ is expressed relative to Vienna Pee Dee Belemnite) and (2) total uptake in µg ^{13}C per µg C copepod per four days [182]. Since the offered diatoms had different initial $\delta^{13}C$ signatures (16235 ‰ for 0 µg/L atrazine, 11326 ‰ for 20 µg/L atrazine and 3217 ‰ for 100 µg/L atrazine), the total uptake per individual was further standardized taking into account the proportion of ^{13}C in each food source, i.e. atomic ^{13}C%. Atrazine concentrations were measured with a mass spectrometer at the start and end of both experiments and additionally before replacing the solutions in the grazer experiment.

Statistics. All experiments used three replicates (see § *Experimental design*). We calculated the mean of these three replicates and used that mean for statistical analysis. Two generalized additive mixed models (gamm) were constructed (in the free software R package 'mgcv') to test if the measured diatom density and Fv/Fm significantly changed with time and atrazine concentration [186]. More information about these methods are available in Text section D2 in Appendix D.

Differences in total fatty acid concentrations in diatom cells and total uptake of ^{13}C enriched diatoms by copepods at the end of the experiment were investigated by comparing means by a one-way ANOVA using the post hoc Tukey HSD for multiple comparisons between control and atrazine treatments. All tests for significance were performed using an alpha level of 0.05.

5.2.2 Modelling the effect of atrazine on a consumer-resource system

Intrinsic growth rate and carrying capacity. Implications of atrazine effects on zoo-phytobenthos dynamics were examined using a Rosenzweig-MacArthur food chain model. The intrinsic rate of increase r and carrying capacity K of *S. robusta* were estimated for each atrazine treatment by fitting a logistic growth model to the observed diatom density dynamics [31]:

$$\frac{dN}{dt} = r \times (1 - \frac{N(t)}{K}) \times N(t) \qquad \text{Equation 5.1}$$

where N(t) represents the diatom cell density as a function of time t [31]. The initial condition (N(t) at t=0) was set equal to the initial density of *S. robusta* in the microcosms. Using the Solver option in Excel, r and K were derived by minimizing the sum of squared differences between the estimated and observed diatom densities.

Next, we examined if the r and K values changed with the atrazine exposure and if this change could be predicted from the atrazine concentration (C) and literature values for the 50% effect concentration for population change (EC_{50}), the lifetime fecundity for the nonexposed diatoms $R_0(0)$ and the concentration-response slope specific for organic chemicals (ß), as described by Hendriks et al. [31]:

$$\frac{r(C)}{r(0)} = 1 - \frac{\ln\left(1 + \left(\frac{C}{EC_{50}}\right)^{-\frac{1}{\beta}}\right)}{\ln(R_0(0))} \qquad \text{Equation 5.2}$$

where r(C) represents the exposed and r(0) the control intrinsic rate of increase. No EC_{50} was found in literature for the tested diatom species, so the values for two taxonomically related species were used. An EC_{50} for *Navicula inserta* of 460.0 µg/L was found for photosynthesis rate reduction after a test period of 1.5 hours [187]. A geometric mean of 64.1 µg/L was derived for the diatom *Skeletonema costatum*, based on EC_{50} values for population change and abundance effects over 2-5 days of atrazine exposure (http://cfpub.epa.gov/ecotox/, [188, 189]). We calculated the predicted effect on the diatom's r using both EC_{50}s. In addition, we derived an EC_{50} for *S. robusta* by calibrating the concentration-response function (Eqn. 5.2) to the inferred r values, again using the least-squares method. Table 5.1 lists all the used parameter values.

Table 5.1 Variables and parameters to estimate the toxic impact of atrazine on the standardized intrinsic rate of increase r(C)/r(0)

Parameter	Description	Value	Unit	Reference
C	Atrazine concentrations experiment	0, 1, 10, 20, 40, 100	µg/L	[a]
β	Concentration-response slope specific for organic chemicals	-0.33	-	[31]
$R_0(0)$	Lifetime fecundity in nonexposed unicellular populations	2	#/ind	[31]
r	Estimated intrinsic rates of increase of *Seminavis robusta* exposed to respectively 0, 1, 10, 20, 40 and 100 µg/L atrazine	0.50, 0.46, 0.52, 0.42, 0.38, 0.36	day^{-1}	[a]
EC_{50}	Effect concentration for 50% of the diatoms			
	Seminavis robusta	162.2	µg/L	[31], [a]
	Navicula inserta [b]	460.0	µg/L	[187]
	Skeletonema costatum [c]	64.1	µg/L	[188, 189], [d]

[a] Derived in the current study.
[b] Maximum value.
[c] Geometric mean of 24, 50, 53 and 265 µg/L.
[d] http://cfpub.epa.gov/ecotox/.

Eqn. 5.2 described the relationship between the chemical concentration and a response on r. We examined whether this model could also be applied to the carrying capacity. Based on an extensive review (Figure 4 in Hendriks et al. 2005) [31], the ratio between the standardized intrinsic rate of increase r(C)/r(0) and standardized carrying capacity K(C)/K(0) was found to be about similar. This relationship between r(C)/r(0) and K(C)/K(0) ratios was confirmed for *S. robusta* by our own experiments (Appendix D, Figure D1). Therefore, we examined if the observed atrazine-induced changes in K could be described by the same relationship as the one used for r (Eqn. 5.2).

Additionally to Eqn. 5.2, a simpler linear model was fitted through the r(C)/r(0) and K(C)/K(0) values to test whether the model fit was closer to the experimental data.

5.2.3 Zoo-phytobenthos oscillations

The long-term implications of the impacts of three atrazine concentrations (0, 100 and 150 µg/L) on the r and K of diatoms in a consumer-resource system were examined using a Rosenzweig-MacArthur model formulation [15]:

$$\frac{dN_1}{dt} = r_1 \times \left(1 - \frac{N_1}{K_1}\right) \times N_1 - \max(k_{n,2}) \times \frac{N_1^{\beta}}{N_1^{\beta} + N_{50,1}^{\beta}} \times N_2 \qquad \text{Equation 5.3}$$

$$\frac{dN_2}{dt} = \left(p_{an,2} \times p_{pa,2} \times \max(k_{n,2}) \times \frac{N_1^{\beta}}{N_1^{\beta} + N_{50,1}^{\beta}} - k_{m,2}\right) \times N_2 \qquad \text{Equation 5.4}$$

The population densities of diatoms (N_1) and its grazers (N_2) were described as a function of time t with parameters linked to body mass m_1 and m_2 [15]. The intrinsic rate of increase r_1 of diatoms differed most between 0 and 100 µg/L atrazine (Table 5.1). We used an atrazine concentration of 150 µg/L to simulate a

higher impact on the zoo-phytobenthos system. The r_1 and the carrying capacity K_1 for 0, 100 and 150 µg/L atrazine exposed diatom populations were determined by multiplying derived r(C)/r(0) and K(C)/K(0) ratios from the best fitting concentration-response model (Eqn. 5.2 or linear) of *S. robusta* with respectively the r(0) and K(0) of nonexposed diatom populations. The fitted values for r(0) and K(0) were compared to default values to evaluate parameter estimation. A description and calculation or typical value of the used variables and parameters in the Rosenzweig-MacArthur model are available in Table 5.2.

Also the maximum consumption rate $\max(k_{n,2})$ was estimated using allometry and this estimate was compared to the rates determined from the grazer experiment. To that end $\max(k_{n,2})$ values were derived from the inferred total uptake of diatoms exposed to 0, 20 and 100 µg/L atrazine (see § Analytic techniques and data treatment). First, the average consumption rate k_n was determined from the total uptake (µg ^{13}C/µg C copepod/four days). A carbon to wet weight fraction for diatoms and copepods was assumed to be equal. Additionally, the total uptake was divided by four to obtain a k_n of the wet weight per unit wet weight copepods per day. Finally, a $\max(k_n)$ was estimated from the relationship between the maximum and average consumption rate k_n as described by Figure 1f in Hendriks et al. 2007 [190]. We derived approximately a factor 5 difference.

To explore the long-term consequences of atrazine exposed phytobenthos to a zoobenthos the period and amplitude of the possible oscillations were determined. The period τ_o is the time in days for one oscillation of diatom and grazer increase and decrease to occur. The amplitude was calculated for each species by dividing the maximum occurring population density by the minimum occurring population density (max(N)/min(N)) [15]. Acknowledging that uncertainty surrounds the allometric relationships we used here, we performed a sensitivity analysis with the free software R [186] to examine the influence of the uncertainty on the allometric coefficients (gamma,n; gamm,p) on model output. To this end, we performed all calculations 100 times, each time randomly sampling each parameter from a uniform distribution with a -20% and +20% deviation from the default estimate as minimum and maximum, respectively (Table 5.2).

Table 5.2 Variables and parameters for Rosenzweig-McArthur model simulations of a zoo-phytobenthos system of which phytobenthos is exposed to atrazine

Symbol [a,b]	Description	Value	Unit [c]	Calculated from / typical value for	Reference
Variables					
N_1, N_2	Density	1.69×10^3, 1.67×10^4	#	$K \approx N = \gamma_N \times m^\kappa$	[15]
Parameters					
β	Slope of Holling type II response for intake of nutrients	1.00	-	grazers	[15]
γ_N	Average density coefficient	1.50×10^6	kg^κ/km^2	cold-blooded animals	[15]
γ_p	Average production coefficient	7.50×10^{-4}	kg^κ/day		[15]
$k_{p,1}$, $k_{p,2}$	Average production rate constant	0.66, 0.07	day^{-1}	$k_p = q_t \times \gamma_p \times m^{-\kappa}$	[15]
$k_{m,2}$	Mortality rate constant	0.07	day^{-1}	$k_{m,2} = k_{p,2}$	[15]
$max(k_{n,2})$	Max. consumption rate constant	5.18×10^{-2}, 3.45×10^{-2}, 3.30×10^{-2}	day^{-1}	uptake *S. robusta* by *D. palustris*. C = 0, 100, 150 µg/L [d]	[e]
"	" " (Default value)	1.14	day^{-1}	$max(k_{n,2}) = (\ln(R_0)+1)/(p_{an,2} \times p_{pa,2}) \times k_{p,2}$	[15]
κ	Allometric exponent	0.25	-	densities scale to species mass	[15, 190]
K_1	Carrying capacity	2.13×10^6, 1.62×10^6, 1.37×10^6	#	C = 0, 100, 150 µg/L in linear regression (Figure 5.2)	[31],[e]
K_1, K_2	" " (Default values)	1.69×10^3, 1.67×10^4	#	$K = \gamma_N \times m^\kappa$	[15]
m_1	Mass	1.62×10^{-12}	kg/cell	geometric mean of five diatom species	[f]
m_2	Mass	1.53×10^{-8}	kg/ind	copepod with body length of 0.6 mm	[f]
$N_{50,1}$	Half saturation density	4.67×10^5, 3.30×10^5, 8.25×10^4	#	$N_{50} = 0.2 \times K_1$ for C = 0, 100, 150 µg/L	[15]
$p_{an,2}$	Fraction assimilated of ingested biomass	0.40	kg/kg	herbivores	[15]
$p_{pa,2}$	Fraction produced of assimilated biomass	0.25	kg/kg	invertebrates	[15]
q_t	Temperature correction for 20 °C	1.00	-		[15]
r_1	Max. intrinsic rate of increase	0.48, 0.34, 0.27	day^{-1}	C = 0, 100, 150 µg/L in linear regression (Figure 5.2)	[31],[e]
"	" " (Default value)	0.46	day^{-1}	$r = \ln(R_0) \times k_{p,1}$	[15]
R_0	Potential lifetime fecundity	2	#/ind	unicellular organisms	[15, 190]
⍰ t ⍙	Time between two measurements	0.10	days		[e]

[a] 1 = diatom, 2 = grazer.
[b] Parameters $max(k_{n,2})$, K_1 and r_1 were derived in the current study and estimated from allometric relationships of Hendriks and Mulder (2012) [15].
[c] Kg is in wet weight.
[d] C is atrazine concentration.
[e] Derived from experiments in the current study.
[f] Values, calculations and references are available in the Appendix, Table D1

5.3 Results

5.3.1 Diatom quantity

The diatom density in the microcosms changed significantly over time, depending on atrazine exposure (gamm; $F(17.46) = 145.9$, $p < 0.0001$). Increased atrazine concentrations led to a decrease of the diatom densities and hence lower overall (biomass) yields in the stationary phase (after approximately 12 days; Figure 5.1). Measured atrazine concentrations differed less than 20% from nominal values (Appendix, Table D2). Therefore, nominal concentrations will be used to refer to the treatments throughout this paper. Atrazine showed to be relatively stable as the initial concentration degraded between 2.4 to 20.0% within four weeks (Table D2). As the concentrations at the end of the grazing experiment were far below the range of 121 to 1000 µg/L LC_{50} for short-term effects on marine harpacticoid copepods, we assume the grazer was not directly affected by atrazine (Table D2) [191, 192].

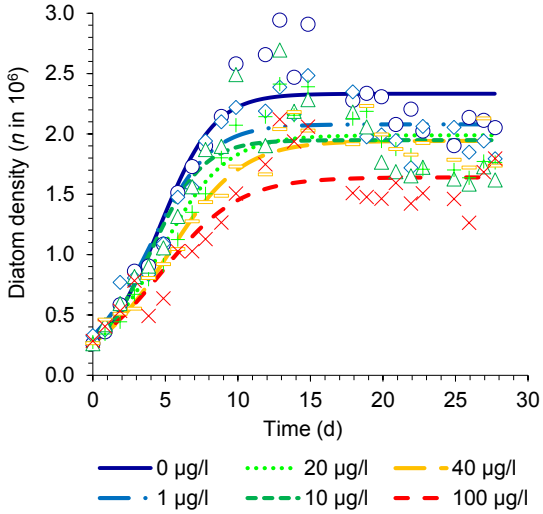


Figure 5.1 Diatom growth curves over a time span of 28 days of exposure to: 0 (○), 1 (◊), 10 (Δ), 20 (+), 40 (–) and 100 (×) µg/L atrazine (nominal concentrations) and the fitted logistic models (lines).

Fitting the logistic growth model for diatoms to the observed diatom densities at various atrazine concentrations yielded standardized intrinsic rates of increase r(C)/r(0) and standardized carrying capacity rates K(C)/K(0) as a function of the atrazine concentrations. Both parameters decreased with increasing atrazine exposure (Figure 5.2). To determine concentration-response models with Eqn. 5.2, EC_{50} values were gathered from literature for the diatoms *N. inserta* and *S. costatum* and determined based on experimentally derived r values for *S. robusta*. The model predicted a lower r and K for *S. robusta* compared to the diatom *N. inserta* and higher values compared to the diatom *S. costatum* when exposed to atrazine (Figure 5.2). The measured responses for r and K of *S.*

robusta were underestimated by the concentration-response model. The linear model fitted the response data better and explained 68% and 75% of the variance in r and K, respectively.

5.3.2 Diatom quality

Atrazine affected the quality of the diatom cells, as indicated by a change in the maximum quantum yield of photosynthetic activity (Fv/Fm) and total fatty acid (FA) concentrations in the cells. The Fv/Fm ratios were significantly lower at higher atrazine exposure, e.g. compared to the control situation a 25% reduction at 100 µg/L atrazine exposure (gamm; $F(17.59) = 11.44$, $p < 0.0001$; see Appendix, Figure D2). Total FA concentrations were significantly different between treatment groups (one-way ANOVA; $F(5,12) = 5.348$, $p \leq 0.01$). A post-hoc Tukey test revealed that total FA concentrations were significantly higher in diatoms exposed to 100 µg/L atrazine (183.5 ± 32.9 µg FA/mg dry weight) compared to diatoms exposed to 0, 1 and 10 µg/L atrazine (respectively, 90.3 ± 14.7, 100.5 ± 38, and 112.1 ± 12.8 µg FA/mg dry weight, $p \leq 0.05$) (Appendix, Figure D3). The FA composition of diatoms was similar in all atrazine treatments (Appendix, Figure D4). Three fatty acids; palmitic acid (16:0), palmitoleic acid (16:1ω7t) and EPA (20:5ω3), accounted for 81-85% of the total amount.

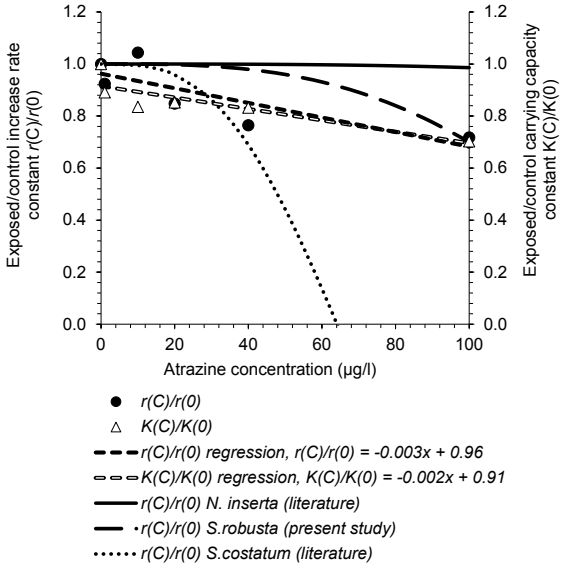


Figure 5.2 The effect of atrazine (µg/L) on the ratio of the exposed and control population intrinsic rate of increase r(C)/r(0) and the ratio of exposed and control population carrying capacity K(C)/K(0) of diatoms. Rate of increase and carrying capacity ratios gained from fitting the logistic growth model to observed *S. robusta* densities (Eqn. 5.1, dots). The ratios estimated with the linear model (open and closed dashed lines) for r and K and estimated with the concentration-response model (i.e. Eqn. 5.2) for a maximum EC50 of the diatom *N. inserta* (460.0 µg/L, solid line), an average EC_{50} of the diatom *S. costatum* (64.1 µg/L, dotted line) and the EC_{50} determined through calibration on inferred intrinsic rates r(C) and carrying capacities K(C) of *S. robusta* (162.2 µg/L, dashed line). One concentration-response function was used for r(C)/r(0) and K(C)/K(0) (Appendix, Figure D1; [31]).

5.3.3 Total uptake of diatoms by copepods

Short-term uptake of diatoms by copepods was not significantly affected when diatoms were exposed to atrazine (one-way ANOVA; $F(2,6) = 0.615$, $p > 0.05$). All copepods survived the grazing experiment, except for one individual in the 20 µg/L atrazine treatment. The measured specific uptakes (µg $\Delta\delta^{13}C$) and total uptakes (µg ^{13}C/µg C after 4 days) are available in the Appendix (Tables D3 and D4).

5.3.4 Zoo-phytobenthos oscillations

Zoo-phytobenthos dynamics for different levels of atrazine exposure were simulated with a Rosenzweig-McArthur model to explore the consequences of atrazine effects on phytobenthos in a consumer-resource system. To this end, we calculated intrinsic growth rate r and carrying capacity K values for diatoms at 0, 100 and 150 µg/L atrazine from the linear models for *S. robusta* (Figure 5.2). Both parameters decreased at increased atrazine exposure (Table 5.2). The r and K of diatoms in control treatments differed respectively a factor 1.04 and three orders of magnitude from allometric estimated values (Table 5.2). Maximum consumption rates were derived from the total uptake of atrazine treated *S. robusta* by *D. palustris*. The $\max(k_{n,2})$ decreased slightly at an increased atrazine exposure to diatoms: 5.18×10^{-2}, 3.45×10^{-2} and 3.30×10^{-2} day^{-1} at respectively 0, 20 and 100 µg/L atrazine. The allometric estimated $\max(k_{n,2})$ of 1.14 day^{-1} differed a factor 22 compared to the measured rate in the control treatment (Table 5.2).

Firstly, zoo-phytobenthos dynamics were simulated with values for the three ecological parameters obtained in the present study: r, K and $\max(k_n)$. The other parameters were set as in Table 5.2. Only diatom populations survived on the long-term with a constant density. Increased atrazine concentrations led to reduced biomass yields in the stationary phase. Secondly, consumer-resource dynamics were simulated with a generic $\max(k_{n,2})$ of 1.14 day^{-1} instead of the measured consumption rates. Diatoms exposed to atrazine led to a stable oscillating zoo-phytobenthos population system for all three herbicide concentrations (Figure 5.3). Higher atrazine concentrations increased the period τ_o of the diatom and grazer oscillations (Figure 5.3). The amplitude max(N)/min(N) of diatom oscillations decreased (for 0, 100 and 150 µg/L atrazine, respectively 54.2, 32.6 and 25.8) and grazer oscillations slightly increased (respectively 4.2, 4.3 and 4.5; Figures 5.3 and 5.4). The sensitivity analysis indicated that changing the allometric coefficients by 20% shifted the phytobenthos and zoobenthos oscillations in time but did not alter the concentration-specific oscillation periods by more than 10% (Figure D5).

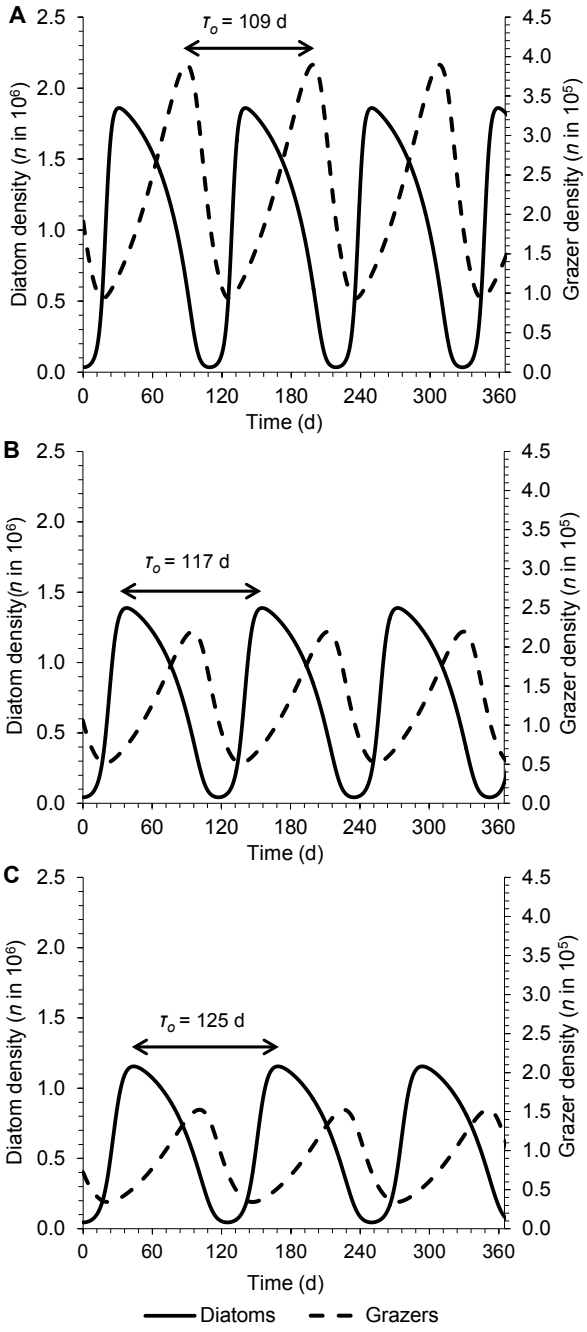


Figure 5.3 Oscillations of the marine benthic diatom population density (resource) and the benthic grazer population density (consumer) simulated over one year with the Rosenzweig-McArthur model (Eqns. 5.3 and 5.4) The diatoms were exposed to A) 0 µg/L, B) 100 µg/L, and C) 150 µg/L atrazine.

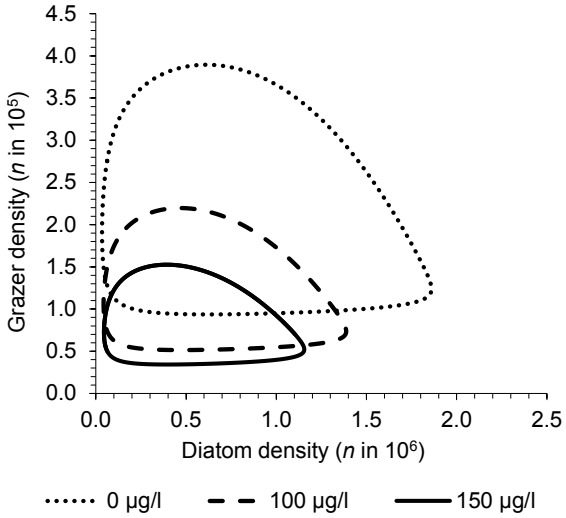


Figure 5.4 Grazer population density versus diatom population density. Diatoms were exposed to 0, 100 and 150 µg/L atrazine.

5.4 Discussion

5.4.1 Diatom quantity

Diatom densities decreased significantly at increased atrazine exposure. The lower overall (biomass) yield in the stationary phase is in line with alterations in freshwater phytoplankton and periphyton communities by atrazine reported in literature [180]. The decrease in diatom density after the stationary phase is commonly observed when nutrient limitation sets in. This phenomenon is sometimes referred to as the declining phase [193].

The established relationship between the standardized r(C)/r(0) and K(C)/K(0) ratios enabled us to use one concentration-response function (Eqn. 5.2) to examine the effects of atrazine on both r and K. Overall, the concentration-response model predicted a decrease in r and K values for *S. robusta*. However, the r(C)/r(0) and K(C)/K(0) ratios of 1 to 40 µg/L atrazine exposed diatoms were below the estimated r(C)/r(0) concentration-response curve for *S. robusta* (Figure 5.2). A qualitative sensitivity analysis of the concentration-response function revealed a better fit of the curve with measured data at a higher ß value (i.e. concentration response slope). Yet, at the same time this diminished the fit at a higher atrazine concentration. Furthermore, the r(C)/r(0) ratios estimated with the concentration-response model were also largely dependent on the species-specific EC_{50} value at high atrazine concentrations. Three diatom species demonstrated reduced r(C)/r(0) and K(C)/K(0) ratios at higher EC_{50} values (Figure 5.2). Therefore, due to uncertainties in the concentration-response model we used the linear regression models to extrapolate r and K responses

above 100 µg/L atrazine for food chain model simulations. These curves fitted the experimental r(C)/r(0) and K(C)/K(0) data quite well as the models explained 68% and 75% of the variation in data.

5.4.2 Diatom quality

The reduced Fv/Fm in diatoms at increased atrazine exposure reflects the toxic mode of action of atrazine, that is inhibiting photosynthesis by blocking electron transport in the Hill reaction of photosystem II [194]. A lower photosynthetic capacity is in line with reduced diatom growth and biomass yield. Five species of marine unicellular algae also demonstrated a relationship between Fv/Fm and biomass reduction in nitrate-limited cultures [195]. Both reduced diatom growth and Fv/Fm may influence grazers by decreased food availability.

The FA concentration in diatom cells, a proxy for nutritional quality for copepods, showed no signs of decrease with increasing atrazine concentrations. Instead, an increase with atrazine exposure was found significant. Sriharan et al. (1989) also revealed an enhancement of lipid production in the diatom *Navicula saprophila* at higher stress levels [196]. In contrast to variations in *N. saprophila* FA composition in a nutrient stress medium, diatom FA composition was unaffected by atrazine in the current study [196]. Three fatty acids (i.e. EPA, palmitic and palmitoleic acid) accounted for 81 to 85% of the total FA concentration, which is in accordance with the 78% reported in a previous study [197]. Some FAs are only produced in primary producers (e.g. 18:2ω6 and 18:3ω3). Consequently, higher trophic species must obtain these so-called essential FAs from their diets [198]. Therefore, a higher FA quantity may be beneficial for the grazers of diatoms. Yet, in the present study, increased FA quantity is combined with a lower diatom density and less initial carbon (^{13}C) in diatoms at higher atrazine concentrations (see section Analytic techniques and data treatment). Less incorporated carbon in diatoms may result in a lower carbon flux from phytobenthos to grazers. Eventually, the advantage of more FA may be cancelled out by a decreased carbon flux and food density.

5.4.3 Modelling atrazine effects on a zoo-phytobenthos system

In the present study, we derived population growth rates, carrying capacities and consumption rates to simulate zoo-phytobenthos dynamics for different levels of atrazine exposure. The empirically derived r of 0.48 corresponded to the allometrically estimated default value of 0.46 for non-exposed diatoms. Empirically and allometrically obtained max(k_n) values of copepods fed with non-exposed diatoms differed by a factor of 22. The empirical max(k_n) of 5.18 $\times$ 10^{-2} day^{-1} only differed within a factor 3 from maximum ingestion rates of the marine copepod *Oithona nana*, the freshwater copepod *Diaptomus gracilis* and the water flea *Daphnia magna* [199]. Yet, experimental conditions, such as exposure time and temperature, and analytical methods differed from the current study (references in [199]). Furthermore, maximum ingestion rates of 17 other

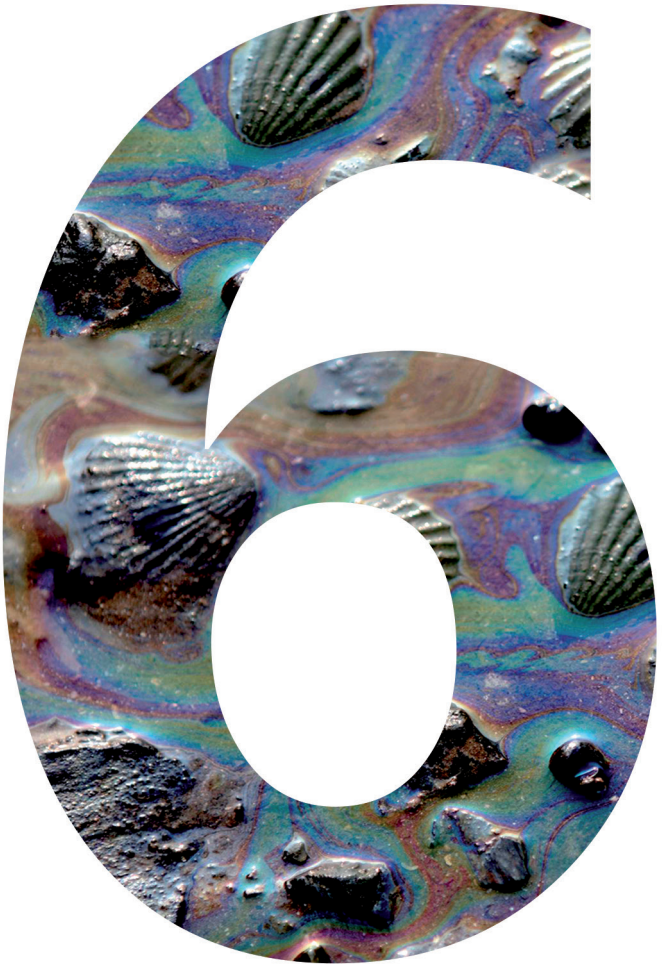
marine and freshwater copepods were between 7 to 160 times higher with an average of 1.67 day^{-1} (range 0.37 to 8.16 day^{-1}) [199]. This average is comparable to the allometrically obtained max(k_n) of 1.14 day^{-1}. Therefore, we simulated zoo-phytobenthos dynamics with the allometric estimated consumption rate as a generic value for copepods.

Periods of both the phytobenthos and zoobenthos population oscillations matched with 100 day periods of freshwater microalgae and copepod *D. magna* grown in laboratory microcosms without additional stress [200]. Max(N)/min(N) ratios for *S. robusta* were on average a factor 2 to 9 higher than the green algae *Chlorella vulgaris* [201]. Max(N)/min(N) ratios of copepods corresponded to amplitudes of the freshwater rotifers *Brachionus calyciflorus* and *Brachionus rubens* and copepod *D. magna* [33, 200, 201]. In the present study, increased oscillation periods (τ_o) of both phytobenthos and zoobenthos corresponded to a lower rate of increase for phytobenthos at increased toxic stress. An atrazine concentration of 150 µg/L caused a 44% reduction of r and a 15% increase of τ_o compared to the control situation. Yearly, the number of oscillations reduced from approximately 3.5 to 3 (Figure 5.3). In a previous study, oscillations of *B. rubens* also reduced in frequency at a higher pentachlorophenol (PCP) exposure and a reduced r [33]. Atrazine exposure of 150 µg/L also caused a 52% reduction of diatom oscillation amplitudes compared to the control situation. In particular, the 36% reduction in carrying capacity of diatoms contributed to this change in max(N)/min(N). Although the effects of 150 µg/L atrazine on the amplitudes of copepod oscillations were small (7% increase), the maximum and minimum copepod density reduced with 61% and 63%, respectively. As such, the food chain model simulations suggest food chain mediated indirect effects on grazer populations. Overall, toxic stress by atrazine will reduce both diatom and copepod availability throughout the year. These patterns have to be confirmed by experimental set ups, despite the challenge to empirically verify consumer-resource interactions without any additional stress factors, especially when the test system is influenced by toxicants.

To our knowledge, this is the first study combining a consumer-resource model with empirically determined growth rate-determining parameters to examine indirect effects of toxic stress via the diet on a marine consumer population. Instead of the initially considered concentration-response model (Eqn. 5.2), a linear model was used to extrapolate atrazine responses on the rate of increase and carrying capacity of diatoms. Yet, the concentration-response model may still be valuable to estimate responses to chemicals of other species than those tested here, thereby reducing the need for toxicity experiments in the future. Although the consumer-resource model is simpler than more realistic marine food web models [202] and its predictions need to be tested using field data, we have demonstrated its value as a hypothesis-generating tool which, in the case of atrazine, suggests that chemical effects on the abundance of a resource can result in altered consumer-resource dynamics.

5.5 Acknowledgements

Frederik de Laender is a postdoctoral research fellow from the Research Foundation-Flanders (FWO). Marleen de Troch is financed by the Special Research Fund of the Ghent University (GOA project 01GA1911W). The authors acknowledge Drs. Clio Cnudde, Ir. Dirk van Gansbeke and Nancy de Saeyer (Ghent University) for their assistance with the lab work. Two anonymous referees gave valuable comments to improve the paper.



Chapter 6
Sensitivity of polar and temperate marine organisms to oil components

Lisette de Hoop
Aafke M. Schipper
Rob S.E.W. Leuven
Mark A.J. Huijbregts
Gro H. Olsen
Mathijs G.D. Smit
A. Jan Hendriks

Published in Environmental Science & Technology 45 (20): 9017-9023

Abstract

Potential contamination of polar regions due to increasing oil exploitation and transportation poses risks to marine species. Risk assessments for polar marine species or ecosystems are mostly based on toxicity data obtained for temperate species. Yet, it is unclear whether toxicity data of temperate organisms are representative for polar species and ecosystems. The present study compared sensitivities of polar and temperate marine species to crude oil, 2-methyl-naphthalene and naphthalene. Species sensitivity distributions (SSDs) were constructed for polar and temperate species based on acute toxicity data from scientific literature, reports and databases. Overall, there was a maximum factor of 3 difference in sensitivity to oil and oil components, based on the means of the toxicity data and the hazardous concentrations for 5 and 50 percent of the species (HC_5 and HC_{50}) as derived from the SSDs. Except for chordates and naphthalene, polar and temperate species sensitivities did not differ significantly. The results are interpreted in the light of physiological characteristics, such as metabolism, lipid fraction, lipid composition, antioxidant levels and resistance to freezing, that have been suggested to influence the susceptibility of marine species to oil. As a consequence, acute toxicity data obtained for temperate organisms may serve to obtain a first indication of risks in polar regions.

6.1 Introduction

The polar regions are subject to contamination supplied by air and water currents emitted in other areas as well as contaminants arising from local activities like tourism, shipping, and infrastructure support for scientific research [203, 204]. Local petroleum-industry activities might also introduce contaminants to the polar environment, notably through produced water discharges and accidental spills [95, 205, 206]. Effluents, produced daily during oil extraction, discharge alkylphenols, metals, organic acids, and oil components, like benzene, toluene, xylene and naphthalene, into the marine environment [95, 207]. In addition, oil spills from shipping and drilling activities occur regularly in marine waters [208]. In future, oil exploitation and transportation in polar regions is likely to increase due to depleting resources elsewhere and increased exploration opportunities due to melting of sea ice [35, 209-211]. Eventually, more petroleum-industry activities will potentially contribute to increased contamination of the polar marine ecosystems [210, 211]. Oil may pose a risk to marine species in polar regions due to its persistence in the environment and its tendency to accumulate in biota [95, 212]. These risks need to be quantified to support environmental decisions to protect polar ecosystems against impacts of pollution [34]. So far, however, regulatory risk procedures and threshold values specific to the polar region are lacking. Even more, it is unclear whether such specific values are needed [205, 213]. Polar risk assessments are mostly based on toxicity data obtained for temperate species. Yet, the question rises whether toxicity data of temperate organisms are representative for polar species and ecosystems [34, 214, 215]. Adaptations of polar organisms to the harsh conditions of the polar environment have been suggested to influence their sensitivity to contaminants [34, 213, 215]. Speculations about differences in sensitivity between polar and temperate marine species are often related to possible differences in physiological characteristics, including their metabolism, lipid fraction and composition, antioxidant defence system and antifreeze capacity [34, 127, 213]. Moreover, characteristics of the polar environment, such as low temperatures and marked seasonality, have been suggested to influence the way contaminants behave [214].

To date, little is known about potential differences in sensitivity to toxicants between polar and temperate species [39]. Moreover, the few studies available do not allow univocal conclusions. For example, the Antarctic sea urchin *Sterechinus neumayeri* was less sensitive to zinc but more sensitive to copper and cadmium than eleven urchin species from temperate regions [216]. Contrastingly, some other polar marine invertebrates, primarily amphipods, were on average equally sensitive to copper and less sensitive to cadmium than numerous temperate marine invertebrates [217]. Five polar marine amphipods were on average equally or less sensitive to zinc and lead than four non-polar marine amphipods [218, 219]. Until now, a comparison of polar and temperate marine species regarding their sensitivity to toxicants other than metals is lacking.

The goal of the current study was to compare the sensitivities of polar and temperate marine species to crude oil and individual oil components. To that end, toxicity data available in scientific literature, reports and databases were collected and Species sensitivity distributions (SSDs; cumulative distribution curves) were constructed for each species group (polar and temperate). Possible differences and similarities in sensitivity to oil and oil components between polar and temperate marine species are discussed in relation to their physiological characteristics.

6.2 Methods

6.2.1 Data collection

A literature search provided 14 articles and reports with toxicity experiments on polar marine species [213, 220-228] and temperate marine species [229-232]. Additional toxicity data on polar and temperate species were obtained from the following databases: RIVM e-toxBase, U.S. EPA ECOTOX, and PAN Pesticide Database [7, 233, 234]. Marine species were considered polar when meeting one or more of the following criteria: 1) mentioned as such in peer reviewed scientific literature; 2) included in the Arctic Register of Marine Species [235]; and 3) a minimum of 75% of the distribution records of the species were located within the Arctic or Antarctic marine region [236]. The temperate species group included marine organisms from the temperate and subtropical climate zones.

Toxicity data comprised acute LC_{50}, EC_{50} and TL_m (median tolerance limit) endpoint values, with mortality or reduced survival effects for 50 percent of the test organisms. Only toxicity data with short term test durations (one to eight days) and salt water test conditions were included. To retrieve sufficient toxicity values, data collection included test results from organisms of different developmental stages, both static and flow through experiments, and both nominal and measured concentrations (respectively 25% and 75% of the 85% available data information). LC_{50} and TL_m values for crude oil were derived from experiments with water-soluble fractions (WSF) of several oil types. The WSF constituents are dispersed particulate oil, dissolved hydrocarbon and soluble contaminants such as metallic ions [237]. WSF is a relatively stable oil-in-water mixture and is, therefore, very useful to assess the toxicity of crude oils for marine organisms [238].

6.2.2 Data treatment

Toxicity data to derive SSDs were available for crude oil and two oil components: naphthalene and 2-methyl-naphthalene (see Appendix E). Two subsets of the crude oil data were used in the SSD construction. One subset consisted of three types of crude oil with similar compositions, i.e. Alaskan North Slope crude oil, Cook Inlet crude oil and Prudhoe Bay crude oil [239]. The other subset consisted

of these three types of crude oil combined with 10 other types, i.e. Artem Island, Kuwait, Neftyany Kamni, Norman Wells, Sangachaly-More, Shengli, South Louisiana and Venezuelan Tia Juana crude oil, and Bunker C and No. 2 fuel oil.

Toxicity data were first $\log_{10}$-transformed. In case of multiple toxicity values for a single substance and a single species, the geometric mean was determined prior to the transformation. For each toxicant and species group (polar and temperate), a sample mean (μ) and standard deviation (σ) were calculated based on the $\log_{10}$-transformed toxicity data. The parameters μ and σ were used in the integral of the normal distribution (the cumulative distribution function) to derive SSDs [21, 240], i.e. the Potentially Affected Fraction (PAF) of species plotted against the environmental concentration of the toxicant. Toxicity is also reported as the hazardous concentration for 5% and 50% of the species (HC_5 and HC_{50}) with a 50% confidence limit [241].

Potential differences in sensitivity between the polar and temperate marine species groups were investigated by comparing the means (μ) and variances (σ) of the $\log_{10}$-transformed toxicity data. If $\log_{10}$-transformed toxicity values from both species groups were normally distributed according to the *Kolmogorov-Smirnov* and *Shapiro-Wilk* tests, the means were compared with the *Independent t-test*. Alternatively, the *Mann-Whitney-U test* was used. The *Levene's test* was used to compare the variances. All tests were executed with SPSS 15.0 for Windows.

6.3 Results

Toxicity values were found for 41 polar species and 49 temperate species. The full dataset is given in Appendix E, Tables E1 and E2. A total of 10 to 28 species were available to derive SSDs for naphthalene, 2-methyl-naphthalene and crude oil (Table 6.1). Species were categorized according to five phyla: Annelida, Arthropoda, Chordata, Echinodermata, and Mollusca. The number of values per taxon differed between the temperate and polar species groups and between the toxicants (Table 6.1). Most data were available for Arthropoda, Chordata and Mollusca, respectively 54%, 32% and 20% for polar species and 49%, 20% and 20% for temperate species.

The SSDs of 2-methyl-naphthalene and crude oil showed little difference between polar and temperate species groups (Figure 6.1). The HC_5 values for 2-methyl-naphthalene and crude oil differed a maximum factor of 1.4 between the two groups, whereas the average toxicities (HC_{50}) were within a factor of 2 (Table 6.2). Only for naphthalene there was a significant difference in both means (p = 0.002) and variances (p = 0.02) of the SSDs, with a factor of 3 difference between the HC_{50} values and a factor 1.2 between the HC_5 values of the temperate and polar species groups (Table 6.2). A comparison of only arthropods, chordates and echinoderms led to a factor of 2 significant difference (p = 0.006) between the polar and temperate naphthalene HC_{50} values (Table 6.2). No significant difference in sensitivity was found between polar and temperate arthropods (*Mann-Whitney-U test*; p = 0.22). The difference between the four polar and four temperate chordates was significant (p = 0.03). The chordates were all fish (Actinopterygii) with exception of the temperate urochordate *Ciona intestinalis*. Comparing only fish resulted in a p-value of 0.06.

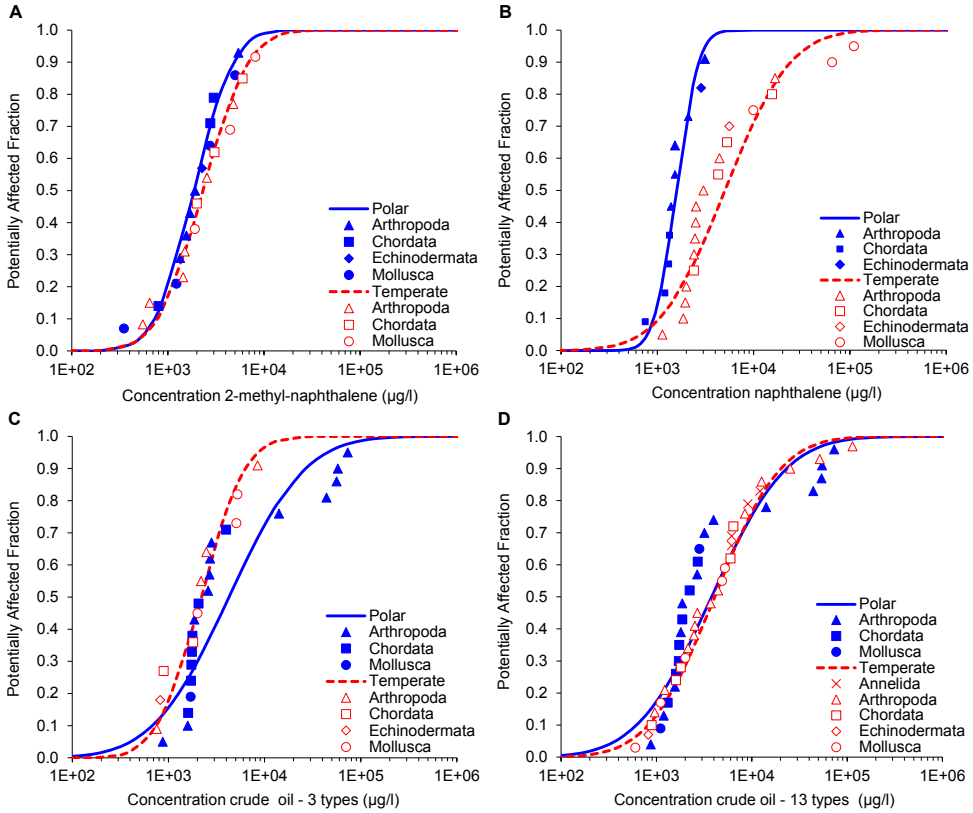


Figure 6.1 Species sensitivity distributions for temperate (dotted curves) and polar (solid curves) marine species for a) 2-methyl-naphthalene, b) naphthalene, c) 3 types of crude oil, and d) 13 types of crude oil.

Table 6.1 Number of temperate and polar marine species, subdivided by phylum, used to derive species sensitivity Distributions for different toxicants

Toxicant	Region	Annelida	Arthropoda	Chordata	Echinodermata	Mollusca	Total
Naphthalene	Temperate	0	11	4	1	3	19
	Polar	0	5	4	1	0	10
2-Methyl-naphthalene	Temperate	0	6	3	0	3	12
	Polar	0	5	3	1	4	13
Crude oil – 3 types	Temperate	0	4	2	1	3	10
	Polar	0	12	7	0	1	20
Crude oil – 13 types	Temperate	4	13	5	1	5	28
	Polar	0	13	7	0	2	22

Table 6.2 Means (μ) and standard deviations (σ) of polar and temperate toxicity data, probability on equality of means and variances between polar and temperate marine species groups (*p*-value), HC_{50} (μg/l) and HC_5 (μg/l) values (50% confidence limit)

		μ [a]	σ [a]	HC_{50} (μg/l)	HC_5 (μg/l)
Naphthalene	Temperate [b]	3.70[d]	0.53	$5.0 \cdot 10^3$	$0.67 \cdot 10^3$
	Temperate [c]	3.53	0.37	$3.4 \cdot 10^3$	$0.85 \cdot 10^3$
	Polar	3.20	0.18	$1.6 \cdot 10^3$	$0.79 \cdot 10^3$
	p-value [b]	0.002	0.02		
	p-value [c]	0.006	0.10		
2-Methyl-naphthalene	Temperate	3.36	0.37	$2.3 \cdot 10^3$	$0.57 \cdot 10^3$
	Polar	3.27	0.32	$1.9 \cdot 10^3$	$0.55 \cdot 10^3$
	p-value	0.49	0.54		
Crude oil – 3 types	Temperate	3.34	0.36	$2.2 \cdot 10^3$	$0.55 \cdot 10^3$
	Polar	3.63[d]	0.62	$4.3 \cdot 10^3$	$0.40 \cdot 10^3$
	p-value	0.47	0.08		
Crude oil –13 types	Temperate	3.61	0.54	$4.1 \cdot 10^3$	$0.52 \cdot 10^3$
	Polar	3.58[d]	0.61	$3.8 \cdot 10^3$	$0.37 \cdot 10^3$
	p-value	0.51	0.59		

[a] Of the $\log_{10}$-transformed LC_{50}, EC_{50} and TL_m values.
[b] Including all taxonomic groups.
[c] Without temperate molluscs.
[d] Toxicity values were not normally distributed according to the *Kolmogorov-Smirnov* and *Shapiro-Wilk tests*.

6.4 Discussion

6.4.1 Uncertainties due to differences in test species

Overall, the results indicate that median hazardous concentrations of polar and temperate marine species for oil and oil components differed less than a factor of 3. The significant difference between the mean sensitivities of polar and temperate species to naphthalene could not be explained by differences in taxonomic groups included in the comparison, as a comparison based on the same taxonomic groups (arthropods, chordates and echinoderms) yielded a significant difference as well. The SSDs for naphthalene suggested that chordates were responsible for the difference between temperate and polar species (Figure 6.1b).

6.4.2 Uncertainties due to test characteristics

Generally, a higher water temperature in ecotoxicological experiments leads to a lower effect concentration (e.g. LC_{50}), thus to a higher sensitivity of an organism to a toxicant [219, 242, 243]. Although water temperatures were reported in only 60% of the literature sources, we found no distinct indication of a bias in the remaining toxicity data. For instance, test conditions of the most sensitive species in the dataset included both high and low water temperatures (see Appendix, Tables E1 and E2). In addition, the 10 species with multiple toxicity and temperature values for a single substance showed no distinct increase or

decrease in effect concentrations with an increasing temperature. For example, LC_{50} values for naphthalene of the pink salmon *Oncorhynchus gorbuscha* both increased and decreased within a temperature rise of 8 °C [244].

Sensitivity to toxicants may vary with life history stages, of which larvae are generally expected to be the most sensitive to oil [245]. Furthermore, static tests may give higher estimates of species sensitivities to oil than flow-through tests [231]. These uncertainties should be assessed when more data become available. The type of concentration probably generated little uncertainty because means and standard deviations of measured concentrations did not differ significantly from data including nominal concentrations.

6.4.3 Comparison with other studies

Crude oil, naphthalene and 2-methyl-naphthalene are expected to exhibit a nonpolar narcotic toxicity mode of action [39, 116, 117, 246]. Nonpolar narcotics penetrate the lipid bilayer region of membranes and thereby alter lipid properties, such as fluidity, thickness, and surface tension, as well as fatty acid composition. Ultimately, the disturbance of the membrane function leads to death of the organism [113, 121, 247]. Based on a generic critical body burden of 0.10 mol/kg wet weight, we derived LC_{50} values for the compounds as described in the Supporting Information (Appendix E1). All measured HC_{50} values were in the same order of magnitude with the estimated LC_{50} values for a narcotic toxicity mode of action of naphthalene and 2-methyl-naphthalene ($5.7 \cdot 10^3$ and $1.4 \cdot 10^3$ µg/l, respectively).

Except for chordates and naphthalene, our comparison of SSDs revealed equal sensitivity to oil for polar and temperate species. This is consistent with a qualitative comparison by McFarlin et al. which showed that three polar species were equally sensitive to one type of crude oil type as seven temperate species [248]. A quantitative comparison of metal toxicity revealed also equal sensitivities to copper and even higher sensitivities of temperate species to cadmium, zinc and lead than polar species [217]. By comparing SSDs for two metals, three pesticides and a narcotic chemical, Kwok et al. found a higher sensitivity of tropical freshwater species than temperate freshwater species [249]. Yet, for other metals the reverse was shown, indicating that there is no consistent latitudinal pattern [249].

6.4.4 Physiological characteristics

Differences in sensitivity to oil between polar and temperate marine species may be influenced by differences in physiological characteristics, including metabolism, lipid fraction, lipid composition, antioxidant levels and resistance to freezing [34, 127, 213]. Generally, the rate of standard metabolism in aquatic organisms decreases two- to threefold with a 10°C decrease in water temperature [190, 242, 250]. While this decrease is likely to reduce uptake [34], elimination

and growth rates are expected to be decreased as well. In addition, the tendency of polar organisms towards gigantism, which causes a lower surface-area-to-volume ratio, has been associated with a reduced contaminant uptake [34]. Reduced uptake because of lower temperatures or larger size may explain why polar organisms have been perceived as more tolerant to short-term toxicant exposure than temperate organisms. Yet, sub-lethal endpoints like growth and reproduction may not be indicative of mortality [34, 217, 245]. In long term toxicity studies on survival, the reduction in uptake may be compensated by a decrease of elimination, yielding similar sensitivities in both types of species. Unfortunately, chronic toxicity data could be obtained for a few polar species only. Furthermore, polar species generally have longer life spans than species from lower latitudes [34, 100, 214]. So, reduced uptake over short periods due to lower temperatures and larger size might be compensated by a more or less proportional decrease of elimination and an increase of life span. Hence, differences in metabolism provide no a priori reason for expecting polar species to be more or less sensitive than temperate species.

Marine polar organisms generally have higher lipid contents than temperate organisms [34, 100, 213]. This has been thought to result in a higher uptake of lipophilic contaminants by polar species, possibly leading to increased sensitivity [34]. Yet, the binding of lipophilic toxicants may leave a smaller amount of oil components to interfere with cell membranes [100]. The larger amount of storage lipids in the Arctic copepod *Calanus glacialis*, for example, was used to explain its lower sensitivity to oil components (PAHs; polycyclic aromatic hydrocarbons) in comparison to the copepod *Calanus finmarchicus* from a lower latitude [100]. Following equilibrium fugacity theory, however, one would expect concentrations in fat tissues to be independent of the fraction of lipids [6]. Therefore, a higher lipid content is expected to have little influence on the susceptibility of marine organisms to oil.

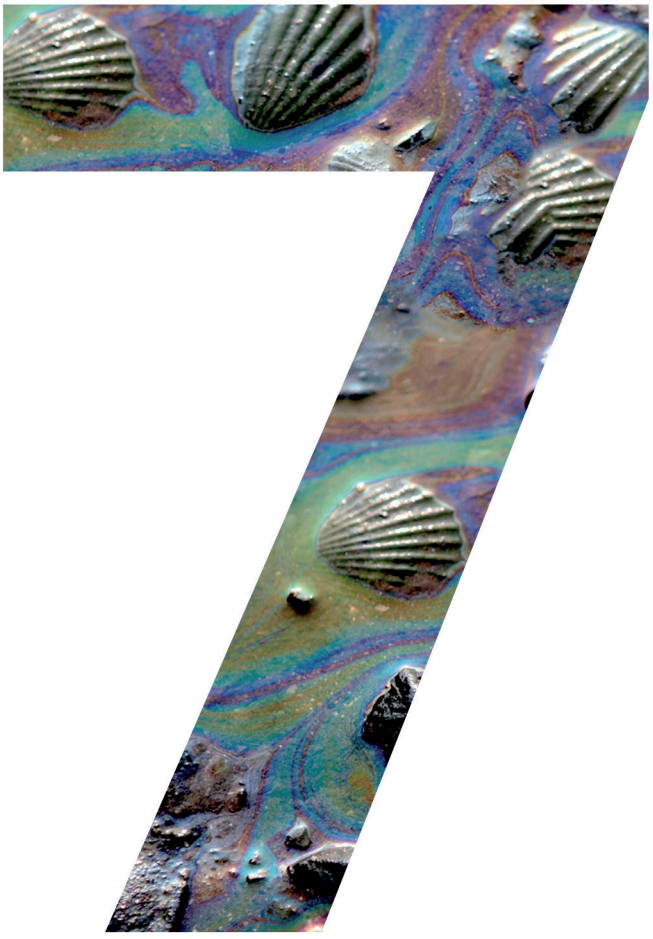
Differences in lipid composition have also been expected to cause a changed sensitivity of polar marine species to oil as compared to temperate species [127]. Lipid composition is thought to influence the distribution of a toxicant in an organism at equilibrium, and thereby the concentration at the target site for nonpolar narcotics, i.e. membranes [127]. Elevated levels of polyunsaturated fatty acids (PUFA) in cell membranes, which appear to be a special adaptation of polar fish and invertebrates to low temperatures [251-253], may indirectly contribute to a higher sensitivity of polar species to oil. PUFA are primary targets for reactive oxygen species (ROS). Additional production of ROS stimulated by the biotransformation of oil constituents taken up by organisms, may lead to an imbalance and thus oxidative damage [252]. Yet, levels of antioxidant enzymes, like vitamins A, C and E, were found to be higher in polar species than temperate species [251, 254]. This may lead to a higher tolerance of polar species to the oxidative effects of oil. With few exceptions [251], this may counteract the negative effects of PUFA in polar cell membranes, possibly leading to equal sensitivities of polar and temperate species.

Finally, the production of antifreeze peptides (AFPs) and antifreeze glycopeptides (AFGPs) is another adaptation to the cold environment noted in several polar species [255, 256] These proteins prevent fish like Polar cod (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*) from freezing by interacting with the cell membranes to protect these against cold damage [257, 258]. It is unknown whether and to what extent the effect of antifreeze proteins on membranes may influence the susceptibility of organisms to oil. The low LC_{50} values noted for *B. saida* for all toxicants (Appendix, Table E1) can be understood from its specialized excretion process to prevent the loss of antifreeze proteins [259]. Due to the lack of glomeruli in the kidneys, toxicants may be excreted via the bile rather than the urine [220, 259]. Excretion via the bile may be disadvantageous, possibly due to a longer retention time of toxicants due to intestinal microflora and reabsorption into the duodenum [259].

Summarizing, our SSDs showed that the sensitivity of polar and temperate marine species to oil and oil components differed on average less than a factor of 3. In addition, most of the differences were not statistically significant and there was no taxonomic group that was consistently more sensitive than the other groups. Apparently, physiological mechanisms suggested to cause differences between polar and temperate species may have little impact on sensitivity to oil. As a consequence, toxicity data obtained for temperate organisms may serve to obtain a first indication of risks in polar regions. Exceptions due to specific mechanisms can be present, however, as noted for example in *B. saida*. In addition, toxicity data on polar species are limited in terms of quantity and quality. Basic conditions, such as temperature have often not been reported. Chronic toxicity data are largely absent. So, more empirical confirmation is definitely needed.

6.5 Acknowledgements

We thank Dick de Zwart and Leo Posthuma for sharing their knowledge on SSDs. We also acknowledge the researchers who answered our questions and provided data. The research was financially supported by Statoil ASA.



Chapter 7
Synthesis

7.1 Introduction

In risk assessment the exposure and effects of chemicals are being assessed to predict the probability and the magnitude of adverse effects on organisms and ecosystems. The aim of this PhD thesis was to evaluate exposure and effect models on their applicability in ecological risk assessment for aquatic species and ecosystems, with a focus on oil constituents. Models were included for different aspects of the risk assessment, namely:

1. a bioaccumulation and a time-varying effect model for a single species,
2. multi-species population models, and
3. a multi-species sensitivity distribution model for polar and temperate communities.

A key question underlying the previous chapters is whether we can use generic models to assess the risk of chemical pollutants in specific systems, such as oil constituents in the Arctic ecosystem. In the present chapter, this question is addressed by evaluating a set of key assumptions (Table 7.1), each representing a possible simplification in exposure and effect modelling employed to assess risks of chemicals in aquatic ecosystems. In section 7.2, the results of the preceding chapters are integrated by discussing these key assumptions. The conclusions and recommendations are given in section 7.3.

Table 7.1 Key assumptions reflecting possible simplifications in exposure and effect modelling employed for risk assessment of chemicals for aquatic species

Model component	Key assumption	Chapters
Bioaccumulation	1. Water is the dominant pathway for uptake of crude oil constituents by cold-blooded aquatic species	2, 3, 4
Single-species effects	2. The influence of biotransformation on internal concentrations of crude oil constituents in crustacean and fish species is negligible	2, 3
	3. A single critical internal threshold is representative for all crude oil constituents in all cold-blooded aquatic species	3, 4, 6
Multi-species effects	4. Migration has a negligible influence on population-level effects of crude oil exposure of copepods	4
	5. Indirect effects of organic chemicals mediated through consumer-resource interactions can be neglected for copepods in dynamic population modelling	5
	6. The sensitivity to crude oil constituents does not differ between polar and temperate marine cold-blooded species	6

7.2 General key assumptions

7.2.1 Key assumption 1. Water is the dominant pathway for uptake of crude oil constituents by cold-blooded aquatic species

Pathways for the uptake of crude oil constituents in aquatic organisms include exposure to contaminated food, water or ingested dispersed oil droplets. In **chapters 2, 3 and 4** only the uptake via the water phase was included in the models. Only a limited number of studies addressed the importance of ingestion for the bioaccumulation of oil constituents in cold-blooded aquatic species. Some studies have examined the transfer of oil constituents from low to higher trophic levels in the aquatic food chain being caused by predation on contaminated food. A laboratory experiment with zebrafish (*Danio rerio*) and cichlids (*Dimidiochromis kiwinge*) showed that the equilibrium internal concentrations of polycyclic aromatic hydrocarbons (PAHs) in these fish only depended on the freely dissolved concentration in water and not on predation between the trophic levels [260]. Moreover, PAHs did not appear to biomagnify in marine food webs in the Baltic Sea, the Tokyo Bay, the Ariake Sea and the Brisbane river estuary [261]. In contrast, however, in Taihu Lake the estimated trophic magnification factor (TMF) for 15 PAHs was higher than one, indicating trophic transfer of PAHs from herbivorous to carnivorous freshwater fish [261]. Further, a modelling study suggested that both aqueous and dietary uptake contributed to benzo(a)pyrene (B*a*P) accumulation in the green mussel (*Perna viridis*), with up to 44% of the total B*a*P accumulation originating from uptake via food [262]. Another modelling study suggested that the dietary uptake of BaP contributed more than 50% to bioaccumulation in copepods and that both uptake via food and water were important for fish [263]. Dietary uptake contributed 21 to 72% to the internal concentration of BaP in mangrove snappers (*Lutjanus argentimaculatus*) depending on the bioconcentration factor (ratio of the chemical concentration in an organism to the chemical concentration dissolved in the water) [263].

In addition to food, the ingestion of dispersed oil droplets has been suggested as an uptake route for oil constituents in aquatic organisms. For example, copepods are known to take up oil droplets and excrete them via faecal pellets [264]. The OMEGA bioaccumulation model has been extended with the ingestion of oil droplets, which increased the model accuracy for the accumulation of individual PAHs and total PAH in the mollusc *Mytilus edulis* [109]. Yet, in the same modelling study the contribution of oil droplets to PAH bioaccumulation was negligible for three fish species and a crustacean [109]. So far, few accumulation models other than OMEGA included the uptake of oil droplets by aquatic organisms. For example, the filtration process of oil droplets from the water column by copepods has been integrated into a model to assess the fate of spilled oil in the marine environment [265]. The study showed that *C. finmarchicus* may filter between 1 and 40% of the spilled oil, depending on the droplet size range model parameter and the spill conditions.

In conclusion, the key assumption that uptake via water is the major contributor to the accumulation of crude oil in cold-blooded aquatic species appears valid for many constituents. Yet, field studies and modelling studies other than with OMEGA did not give a univocal conclusion. For some specific oil constituents both water and food should be taken into account as an uptake route for bioaccumulation. For example, food can largely contribute to BaP accumulation, one of the higher-weighted PAHs, in several aquatic organisms. So far, the ingestion of oil droplets appears a negligible uptake pathway of oil constituents in aquatic organisms.

7.2.2 Key assumption 2. The influence of biotransformation on internal concentrations of crude oil constituents in crustacean and fish species is negligible

In general, biotransformation is a defence or activation mechanism that converts parent compounds into 1) less harmful metabolites that can be excreted from the organism, or 2) more harmful metabolites that contribute to the bioaccumulation in the organism. Particularly organisms that have an active biotransformation enzyme system transform lipophilic organic chemicals generally into more water soluble products that can be readily excreted via urine [23, 80]. Most kinetic models therefore include biotransformation of organic chemicals as an additional elimination route, because biotransformation is expected to reduce the exposure of an organism to the parent compound [2, 95].

In **chapter 2** the OMEGA model was used to evaluate the influence of biotransformation on the accumulation of oil constituents in aquatic species. It was found that the model accuracy increased considerably for fish exposed to PAHs. This is consistent with previous studies that showed fish to biotransform PAHs, such as B*a*P, fluoranthene, and benzo(a)anthracene by using the cytochrome P450 enzymes [87, 88, 208]. Biotransformation rates for fish were therefore also included in the time-varying effect assessment in **chapter 3**, based on quantitative structure-activity relationships (QSARs) [81]. For invertebrates, however, no QSAR was available to adequately quantify the biotransformation of organic chemicals. Although some papers report on biotransformation in aquatic invertebrates, data on actual in vivo rates are scarce [87]. For example, some laboratory studies reported metabolites that were found in vivo after administering PAHs to marine copepods, shrimps, lobsters, crabs and a worm, but these studies did not report quantitative data on transformation rates [50, 266, 267]. It was stated that crustaceans can vary widely in their capacity to metabolize PAHs, yet fish generally have a higher biotransformation rate than marine crustaceans [88, 115, 266]. Additionally, a laboratory-scale food chain study consisting of microalgae, mussels, and fish showed that BaP and 7,12-dimethyl-benz(a)anthracene accumulated more in mussels than in sea basses and this was attributed to a difference in biotransformation capacity [268]. Furthermore, crustaceans have been suggested to metabolize xenobiotics faster than molluscs [87, 88].

In conclusion, biotransformation could influence the bioaccumulation of oil constituents in some crustaceans, though to a lesser extent than in fish. A QSAR for biotransformation rates can be used when modelling the bioaccumulation of organic chemicals for fish. So far, no QSAR has been developed for crustaceans due to data scarcity.

7.2.3 Key assumption 3. A single critical internal threshold is representative for all crude oil constituents in all cold-blooded aquatic species

The critical body burden (CBB) concept states that an organism is adversely affected by a chemical if the body burden exceeds a critical internal threshold level [5]. In this thesis a single CBB (concerning 50% mortality) was assumed to be applicable for risk assessment of all crude oil constituents in all cold-blooded aquatic species and to be independent of exposure time. The relation of a single CBB to chemicals and exposure time are addressed in this paragraph.

Chemicals

The CBB concept is often used for assessing the toxicity of organic chemicals with a narcotic toxic mode of action (TMoA) [2]. This so called baseline toxicity is believed to be the result of nonspecific disturbance of membrane integrity and functioning due to partitioning into biological membranes [14, 116]. The majority of crude oil constituents, such as PAHs, have been suggested to exhibit a narcotic TMoA based on their chemical structure consisting mainly of carbon and hydrogen [117]. As the CBB is expected to be approximately constant for chemicals with the same TMoA, a single CBB (concerning 50% mortality) was assumed to be applicable to all crude oil constituents [116].

Parent chemicals. Results in **chapter 6** endorse the single CBB concept for some oil constituents, as naphthalene and 2-methyl-naphthalene were shown to exhibit their acute lethal toxicity via narcosis in aquatic species as arthropods, fish, molluscs and echinoderms. Yet, these are only two of the many constituents that form crude oil. For certain parent oil constituents the body burden that produces a lethal effect is lower than the narcotic CBB, indicating a more specific TMoA [3]. For example, the mechanism of photoinduced toxicity can lower the CBBs (i.e. enhance the toxicity) for PAHs like fluoranthene and different petroleum mixtures. Ultraviolet light can activate the molecules into an excited state which leads to the formation of the highly reactive, excited single state oxygen that can damage the organism's internal structures [269, 270].

Metabolites. In **chapter 3** metabolic products were excluded from the bioaccumulation modelling of crude oil constituents in fish, mainly because the metabolites were assumed to be less toxic and more susceptible to excretion than the parent compound, which is typical for narcotic chemicals [271]. Only a few metabolites of oil constituents have been identified with a more specific TMoA that could induce detrimental effects at lower concentrations than narcotic

chemicals. For instance, some metabolites of phenanthrene can cause toxic (chronic) effects by a nonnarcotic and nonphototoxic mode of action in juvenile fish [123]. BaP and its metabolic products are known carcinogens [80, 272]. The multicomponent damage assessment model (MDAM) has been developed to analyse the time-dependent toxicity of a mixture with potentially dissimilar TMoAs and toxicokinetic interactions, such as biotransformation [98, 273]. The metabolites' contribution to pyrene toxicity was negligible, whereas metabolites significantly contributed to the toxicity of fluorene to the amphipod *Hyalella azteca* [98]. This model, however, requires toxicokinetic and toxicodynamic parameters for parent compounds and their metabolites. Hence, it will take a lot of effort to parameterize this model for a complex mixture like crude oil.

Mixtures. The total internal concentration of baseline toxicant mixtures can be addressed by the sum of the body burdens of the individual chemicals, which corresponds to the concentration addition model of mixture toxicity [14, 112, 116, 141]. In **chapter 4** a narcotic TMoA, and hence concentration additivity, was assumed for the 25 components representing hydrocarbon blocks that constituted a crude oil. This is in accordance with a study where gasoline hydrocarbons were successfully grouped into hydrocarbon blocks with narcotic TMoA to estimate the toxicity of gasoline to algae in lethal-loading experiments [141]. Another study validated the concentration addition model for narcotic lethal effects of MAHs and PAHs by showing that mortality occurs when a species-specific CBB of any mixture of these aromatics is reached [274]. Finally, a study indicated that mixtures containing approximately 30 or more narcotic chemicals most likely conform to concentration addition [275].

Exposure time

In **chapter 3** the CBB concept was followed to apply one generic lethal body burden (LBB) for estimating time-varying effects of aromatic hydrocarbons on crustaceans and fish. However, differences between the estimated and measured time-varying survival pointed at possible deviations from the assumption of a single CBB. Several other studies have also shown time-dependency of LBB values for narcotic compounds (e.g. PAHs and chlorobenzenes) and for reactive and receptor-mediated compounds in fish, amphipods and crabs [26, 27]. In these experiments it was assumed that the distribution of individual tolerance and toxicokinetics parameters (uptake and elimination rates) was the same among treatments. For longer exposures, the toxicity time course of PAHs in these studies was not only determined by the uptake and elimination of chemicals, but also by a toxicodynamic factor. The time-dependency of the LBB might for instance also be explained by a shift in the TMoA, increased stress or cumulative toxicity with increasing exposure time or by a time-delay between reaching the LBB and death [26, 128]. It has also been suggested that the closer the exposure duration to the life expectancy of the organism, the smaller the CBB for 50% mortality [3].

In conclusion, the assumption of a single CBB, which is independent of exposure time and applicable to all crude oil constituents, is most likely too generic. The simplification of crude oil into 25 hydrocarbon blocks with a narcotic TMoA, and hence the addition of the corresponding body burdens, has not been refuted for toxicity assessments of a crude oil mixture. Yet, for specific oil constituents the TMoA of a parent compound or metabolite differs from narcosis. It is not straightforward to quantify the contribution of these chemicals to the effect of a complex mixture like crude oil. The exact composition of a crude oil is usually unknown. Furthermore, whether or not the conversion of parent compounds to metabolite products leads to a reduced impact of the oil constituent on the organism depends on the TMoA of the metabolite.

7.2.4 Key assumption 4. Migration has a negligible influence on population-level effects of crude oil exposure of copepods

The migration of individuals can be included in population models as a process that modifies the influence of the effect of chemical exposure on simulated changes in overall population biomass. In **chapter 4** the complex 3D multistage SINMOD population model and the simpler consumer-resource Rosenzweig-MacArthur population model were compared for estimating the effects of crude oil on the marine copepod *C. finmarchicus*. In both models the copepods were exposed to the same fluctuations in crude oil concentrations. The migration of individuals was not included in the simple model simulations as the copepod population was 'contained' in an isolated water mass. In contrast, the complex model accounted for migration by including advection processes of the copepods over space and time. Subsequently, this caused a dilution, and thus a decrease, in the amount of crude oil accumulated by the entire copepod population in the complex model. The adverse effect on copepod biomass near the oil spill location was therefore larger when using the simple model compared to the complex model when assuming average and high species sensitivity to crude oil. Both models simulated a negligible effect assuming low species sensitivity to crude oil.

Only few oil spill models have addressed the impacts on organisms and ecosystems as most models focus on the abiotic fate of crude oil [276]. In addition, migration is hardly ever included in dynamic population models that assess chemical impacts on aquatic species. Recently, the fate SINTEF OSCAR model has been coupled with an individual-based model (IBM) for Northeast Arctic cod (*G. morhua*) to evaluate the effects of the time and location of an oil spill on the degree of oil exposure of eggs and larvae [277]. In this study the larval vertical behaviour was included, but not the horizontal drift of individuals. The results demonstrated that the mean egg and larval exposures for cod from different spawning grounds are highly dependent on different the vertical distribution of the offspring and oil [277].

In conclusion, migration of individuals can be an important modifying factor for population-level effects of chemicals. Besides the movement of water, migration can cause a shorter chemical exposure period for organisms in open systems compared to closed systems, thereby leading to a reduced crude oil impact on populations. If this process is not included in simple population models, the extrapolation from individual effects to field populations will be inaccurate due to the overestimation of chemical accumulation in organisms.

7.2.5 Key assumption 5. Indirect effects of organic chemicals mediated through consumer-resource interactions can be neglected for copepods in dynamic population modelling

Apart from direct toxic effects, chemicals may have indirect ecological effects by reducing the amount of food (prey). Indirect toxic effects of a chemical on a copepod via its food were assessed in **chapter 5**. Short-term experiments were combined with food chain modelling to explore the long-term effects of the chemical stressor atrazine on the consumer-resource dynamics of a marine copepod (*D. palustris*) and a diatom (*S. robusta*) species. The model simulations suggested food chain-mediated indirect effects on copepod populations via the reduction in food. This is in accordance to other studies that suggested a reduction in the abundance of consumers because of food limitation due to chemical exposure [278].

However, indirect bottom-up effects via food availability on copepods might only be important for risk assessment in specific cases, for example if the copepod is less sensitive to direct chemical exposure than its prey. This can occur when a chemical exhibits a specific toxicity towards the prey, such as the herbicide atrazine in **chapter 5**. If a chemical (mixture) exhibits a baseline narcotic toxicity, like has been assumed for many crude oil constituents (key assumption 3), both phytoplankton and zooplankton are probably directly affected during chemical exposure. This makes it difficult to distinguish if any effects are also caused indirectly by a decrease in prey or predator abundance.

In addition to bottom-up effects, chemicals can also induce changes in behaviour, competition and grazing rates which can alter species abundances [278]. Several experimental studies showed top-down effects, i.e. an increase in food abundance when grazers were adversely affected by chemicals. For example, microcosm, mesocosm and experimental pond studies showed that a reduced zooplankton abundance due to exposure to benzene, #2 fuel oil and crude oil resulted in an increase in phytoplankton abundance [278-282]. Such top-down trophic level effects of chemicals were also addressed in an ecosystem modelling study that formulated theoretical expectations for ecological effects and recovery for different exposure and ecological scenarios in a shallow pond [167]. A decrease in zooplankton abundance due to chemical exposure caused phytoplankton blooms, which subsequently stimulated zooplankton density during the recovery phase. These effects were also observed for copepod and diatom simulations by using the consumer-resource population model in **chapter 4**. De Laender et al.

(2015) mentioned that, when most abundant species are sensitive to a chemical, indirect effects will be larger than when less abundant functional groups are most sensitive. Direct effects on highly abundant zooplankton might therefore cause indirect effects on phytoplankton in both simple food chains and more complex food webs [167]. Competitive release (removal of competitors) may also indirectly affect species abundance. Yet, distinguishing between competition and trophic-level impacts can be difficult, as few experiments have been designed to isolate the mechanisms causing indirect effects [278].

In conclusion, chemicals can indirectly influence the abundance of copepods or its food via consumer-resource interactions. Effects via food availability will only be important in dynamic populations models if the copepod species is less sensitive to a chemical than its prey. For example, this can occur if a chemical has a specific TMoA for phytoplankton and not for zooplankton. Top-down chemical effects, such as an increase in prey abundance due to reduced predator or grazer abundance can eventually influence the recovery phase of copepods. This should be taken into account in the risk assessment of copepods.

7.2.6 Key assumption 6. The sensitivity to crude oil constituents does not differ between polar and temperate marine cold-blooded species

Risk assessments for polar (Arctic and Antarctic) species and ecosystems are mostly based on toxicity data obtained for species from temperate regions. In **chapter 6**, species sensitivity distributions (SSDs) of measured LC_{50} values were used to compare average sensitivities of polar and temperate marine species. There was a maximum factor of 3 difference in sensitivity to crude oil, naphthalene, and 2-methylnaphthalene. Except for naphthalene, polar and temperate species sensitivities did not differ significantly. A recent study obtained similar results with SSDs of marine Arctic and temperate species exposed to artificial produced water (with oil) [283]. Overall, there were no differences in individual sensitivities and there was no clear pattern of specific taxonomic groups being more or less sensitive than others [283]. Furthermore, an experimental study with dispersed crude oil showed an insignificant difference in sensitivity between Arctic and non-Arctic marine species [284]. In contrast, the results of three experimental studies revealed that the sensitivities of marine copepods to oil exposure differed significantly among geographical zones [285-287]. The sensitivity of copepods to water accommodated fractions (WAF) and water soluble fractions (WSF) of oil increased with increased water temperature [285-287]. Nevertheless, there are also examples of the opposite response [288]. Differences in sensitivity between polar and temperate marine organisms and communities were also recognized in a Norwegian research program, yet, it was shown that the differences were small and could go in both directions [289]. Further, Camus et al. (2015) observed no significant difference in hazardous concentrations for 5% of species (HC_5) and 50% of species (HC_{50}) of produced water (with oil) between temperate and Arctic species by using SSDs of chronic no effect concentration data [283].

In summary, research results so far provide evidence that polar organisms and organisms from temperate regions do not differ in their sensitivity to oil contamination. This suggests that toxicity data obtained for organisms from temperate regions may serve to assess chemical risks in polar regions. Nevertheless, it is recommended to keep in mind that the physiological and biological differences between polar and temperate systems are usually not addressed in standard laboratory toxicity tests [283]. Variation in factors such as the climate, seasonal feeding, distribution of populations and degradation of oil could still induce different responses of polar and temperate ecosystems to oil contamination [283, 289].

7.3 Conclusions

The general key question underlying the previous chapters is whether we can use simple, generic models to assess the risk of mixtures of specific chemical pollutants in specific systems, such as crude oil constituents in the Arctic ecosystem. The current chapter showed that a simple model for risk assessment of crude oil constituents at least has to include uptake via water (key assumption 1), biotransformation factors for fish and possibly for crustaceans (key assumption 2), and a simplified migration process (key assumption 4) (Table 7.2). There are no indications to differentiate between temperate and polar species in constructing an SSD for oil constituents (key assumption 6). Furthermore, trophic bottom-up effects of organic chemicals are probably only important for risk assessment of chemicals with a specific toxicity on basal species such as phytoplankton, for example herbicides (i.e. atrazine), and not for narcotic chemicals with a generic baseline toxicity (key assumption 5). It is probably not necessary to include CBBs for toxic modes of action other than narcosis when crude oil is divided into several hydrocarbon blocks (key assumption 3). Nevertheless, a single CBB for narcosis is not always applicable when assessing risks for individual crude oil constituents. Some constituents exhibit a more specific toxicity than narcosis and the assumption of a single CBB at different exposure times is probably too generic (key assumption 3).

Table 7.2. Summary of the evaluation of various key assumptions representing possible simplifications in exposure and effect modelling employed for risk assessment of chemicals in aquatic ecosystems

Key assumption	Conclusion	Model has to include
1. Water is the dominant pathway for uptake of crude oil constituents by cold-blooded aquatic species	Water can be a dominant pathway, but not for all oil constituents	At least uptake via water
2. The influence of biotransformation on internal concentrations of crude oil constituents in crustacean and fish species is negligible	Biotransformation is not negligible for fish and some crustaceans	Biotransformation in fish and possibly crustaceans
3. A single critical internal threshold is representative for all crude oil constituents in all cold-blooded aquatic species	No single value can be used for all crude oil constituents; only for crude oil if mixture is divided into simplified hydrocarbon blocks	Critical body burdens that are distinctive between different toxic modes of action of chemicals and exposure time
4. Migration has a negligible influence on population-level effects of crude oil exposure of copepods	Migration is an important modifying factor as it can shorten the chemical exposure period	Accounting for migration of individuals
5. Indirect effects of organic chemicals mediated trough consumer-resource interactions can be neglected for copepods in dynamic population modelling	Indirect effects via food availability only important for specific toxicants; top-down effects can be important for the recovery phase of the consumer	Bottom-up trophic consumer-resource effects for specific toxicity, not for baseline toxicity
6. The sensitivity to crude oil constituents does not differ between polar and temperate marine cold-blooded species	So far, the sensitivity does not differ	No differentiation between temperate and polar species

7.4 Recommendations

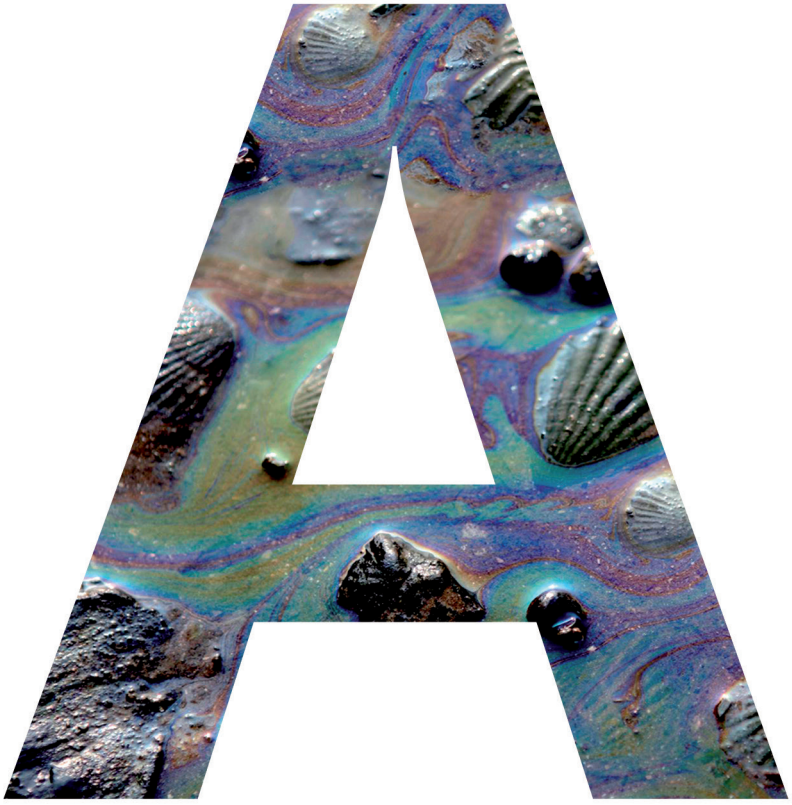
Several improvements are suggested for future usage of the generic and simple bioaccumulation, effect and population models evaluated in this thesis.

1. It is recommended to include more explanatory variables for toxicokinetics, such as biotransformation rates and molecular mass of the chemicals, to improve the performance of the OMEGA model. Biotransformation could be included as an additional elimination route for fish. The efforts needed to include biotransformation in estimations for crustaceans might not offset the benefits in terms of model performance, but this should be tested for a few more crustacean species and crude oil constituents to be certain.

2. It is recommended to further investigate the validity of the CBB concept for the effect assessment of crude oil constituents. This includes whether multiple CBBs should be produced that are distinctive between oil constituents with dissimilar TMoAs and between different exposure times. An investigation into the underlying mechanisms for these differences should give more clarity on the changes that are needed to improve the effect estimates of the OMEGA model.

3. It is recommended to include a migration factor in simple population models that are used for ERA of crude oil. Additionally, it is recommended to validate oil spill models that address the impact on biota with field data on species abundance and oil concentrations before and after the event of an oil spill. In the near future, field data could perhaps be generated in controlled outdoor oil spill experiments, which would provide a better insight in short-term and long-term effects of crude oil on aquatic communities in the field.

4. Overall there may be little differences in species sensitivity between the temperate and polar regions, but exceptions due to different modifying factors can be present. Toxicity data on polar species are limited in terms of quantity and quality. When more data become available, species sensitivity differences could be tested using SSDs based on acute and chronic external and internal toxicity data for more oil constituents, species, and different organism life stages. Additionally, possible underlying mechanisms for these differences, due to modifying factors as physiological mechanisms and external climate factors, should be investigated.



A
Appendix to chapter 2

Table A1 Dry to wet weight ratios for several aquatic species

Species scientific name	Species common name	Phylum	Dry to wet weight ratio	Reference
Chlorella fusca var. *vacuolata*	Green algae	Chlorophyta	0.10	[11]
Selanastrum capricornutum	Green algae	Chlorophyta	0.10	[11]
Mytilus edulis spp.	Blue mussel	Mollusca	0.19	[290]
Lepomis macrochirus	Bluegill	Osteichthyes	0.22	[291]
Oncorhynchus mykiss	Rainbow trout	Osteichthyes	0.25	[11]
Pimephales promelas	Fathead minnow	Osteichthyes	0.18	[292]
Platichthys stellatus	Starry flounder	Osteichthyes	0.25	[11]

A1. The OMEGA model

The absorption rate constant $k_{0,in}$ (L/kg·day^{-1}) was calculated according to [6]:

$$k_{0,in} = \frac{w^{-\kappa}}{\rho_{H_2O,0} + \frac{\rho_{CH2,i}}{K_{ow}} + \frac{1}{\gamma_0}} \quad \text{Equation A1}$$

where w represents the species wet weight, ρ the resistances of a chemical to permeate through lipid layers ($\rho_{CH2,i}$) and to diffuse through water layers ($\rho_{H2O,0}$). In addition the influx is limited by a delay in the flow of water through the organism ($1/\gamma_0$) [6]. The κ, i, and K_{ow} are explained in Table A2.

The total elimination rate constant $\Sigma_{kj,out}$ (day^{-1}) is the sum of three rate constants for minimum elimination via excretion with water (Eqn. A2), egestion with faeces (Eqn. A3) and dilution by biomass as a consequence of growth or reproduction (Eqn. A4) according to [6]:

$$k_{0,out} = \frac{1}{p_{CH2,i} \times (K_{ow} - 1) + 1} \times \frac{w^{-\kappa}}{\rho_{H2O,0} + \frac{\rho_{CH2,i}}{K_{ow}} + \frac{1}{\gamma_0}} \quad \text{Equation A2}$$

$$k_{1,out} = \frac{1}{p_{CH2,i} \times (K_{ow} - 1) + 1} \times \frac{w^{-\kappa}}{\rho_{H2O,1} + \frac{\rho_{CH2,i}}{q_T \times K_{ow}} + \frac{1}{p_{CH2,i-1} \times K_{ow} \times (1 - p_1) \times q_T \times \gamma_1}} \quad \text{Equation A3}$$

$$k_{2,out} = q_T \times \gamma_2 \times w^{-\kappa} \quad \text{Equation A4}$$

Here, p represents the lipid fraction of the organism ($p_{CH2,i}$) and its food in the gut ($p_{CH2,i-1}$), q_T the correction factor of rate constants for temperature dependence, p_1 the fraction of ingested food that is assimilated, γ_1 and γ_2 the rate constants for food consumption and biomass (re)production, respectively. The conceptual diagram (A1) shows an overview of the absorption and elimination rates in the OMEGA model.

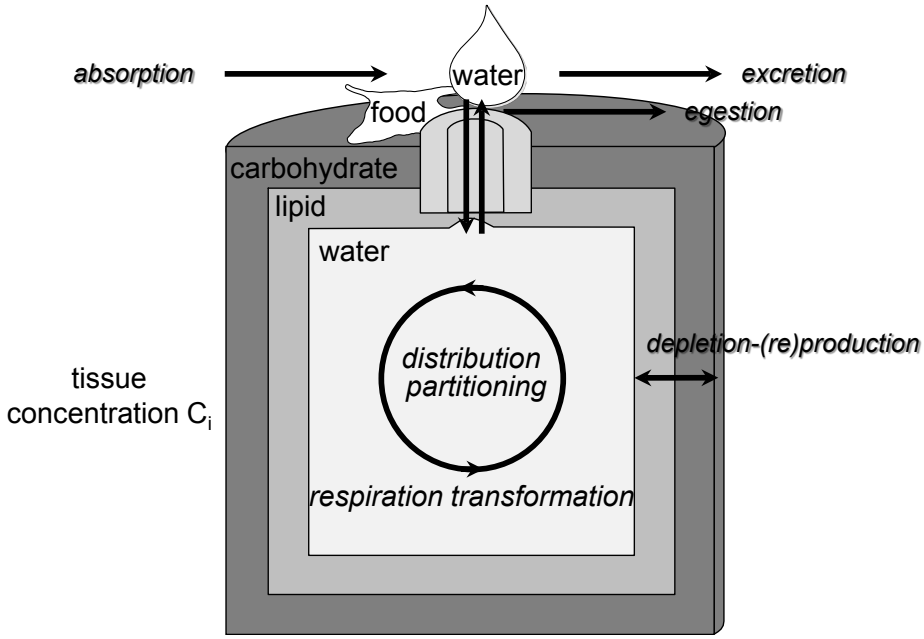


Diagram A1. The internal chemical concentration in an organism is estimated based on the uptake (absorption) and elimination rate constants of the chemical (excretion with water, egestion with faeces and dilution by biomass as a consequence of growth or reproduction). The concentration in the tissue of the organism is determined by the lipid and water layer resistance as well as by the metabolic flows that carry substances into and out of organisms [104].

Table A2 Parameters and variables used for estimating absorption and elimination rate constants and bioconcentration factors with the OMEGA bioaccumulation model

Symbol	Description	Unit [a]	Typical value for/calculated from	Reference
i	Trophic level [b]		-2 = detritivores; -1 = unicellular organisms; 1 = algae; 2 = herbivores; 3 = carnivores	[c]
j	Medium		0 = water, 1 = food, 2 = biomass	[6]
$C_{0,w}$	Concentration in water	µg/L	Variable	[6]
C_i	Concentration in organism	µg/kg	Variable	[6]
K_{ow}	Octanol-water partition ratio	-	EPI Suite	[75]
w	Species weight	kg	Empirical value	[c]
$p_{CH2,i}$	Lipid fraction of species	$kg \cdot kg^{-1}$	Empirical value, otherwise default of 0.01 (algae and unicellular organisms), 0.03 (annelids, arthropods, molluscs), or 0.05 (fish)	[c, 113]
$p_{CH2,i-1}$	Lipid fraction of food	$kg \cdot kg^{-1}$	Fraction of food of trophic level -1 and 1 = 0, -2 and 2 = 0.01, 3 = 0.03	[c]
κ	Rate exponent		0.25	[6]
$\rho_{H2O,j}$	Water layer diffusion resistance	$day \cdot kg^{-\kappa}$	$2.8 \cdot 10^{-3}$ (j = 0), $1.1 \cdot 10^{-5}$ (j = 1)	[6]
$\rho_{CH2,i}$	Lipid layer permeation resistance	$day \cdot kg^{-\kappa}$	$4.6 \cdot 10^{3}$ (i = -1 and 1), $6.8 \cdot 10^{1}$ (i = -2 and ≥ 2)	[6]
p_1	Fraction ingested food assimilated	$kg \cdot kg^{-1}$	0 (i = -1 and 1), 0.2 (i = -2); 0.4 (i = 2); 0.8 (i = 3)	[6]
q_T	Temperature correction factor	$kg \cdot kg^{-1}$	1 for cold-blooded organisms	[6]
γ_0	Water absorption-excretion coefficient	$kg^{-\kappa} \cdot day^{-1}$	200 (water breathing organisms)	[6]
γ_1	Food ingestion coefficient	$kg^{-\kappa} \cdot day^{-1}$	0 (i=1 and -1), $5.0 \cdot 10^{-3}$ (i = -2 and ≥ 2)	[6]
γ_2	Biomass (re)production coefficient	$kg^{-\kappa} \cdot day^{-1}$	$6.0 \cdot 10^{-4}$ (All organisms)	[6]

[a] Kg is in wet weight.
[b] Detritivores are annelids, insects and crustaceans (i.e. *Asellus* sp., *Gammarus* sp., *Portunus* sp.). Unicellular organisms are bacteria, ciliates, diatoms and rotifers. Herbivores are crustaceans, molluscs and fish. Carnivores are fish.
[c] Collected in the current study.

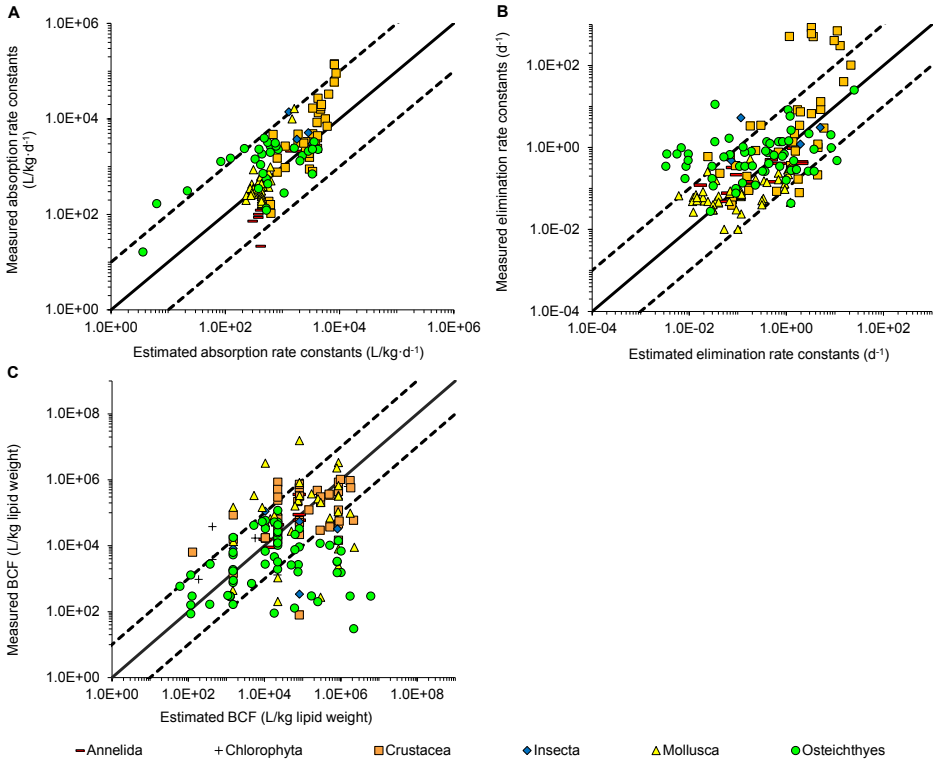


Figure A1 Geometric mean for A) absorption rate constants, B) elimination rate constants, and C) lipid-normalized bioconcentration factors (BCF) of organic constituents measured in experiments versus the geometric mean estimated with the OMEGA bioaccumulation model for aquatic species from different taxonomic groups. The 1:1 line indicates a perfect model fit. The dashed lines represent a factor of 10 under- and overestimation by OMEGA.

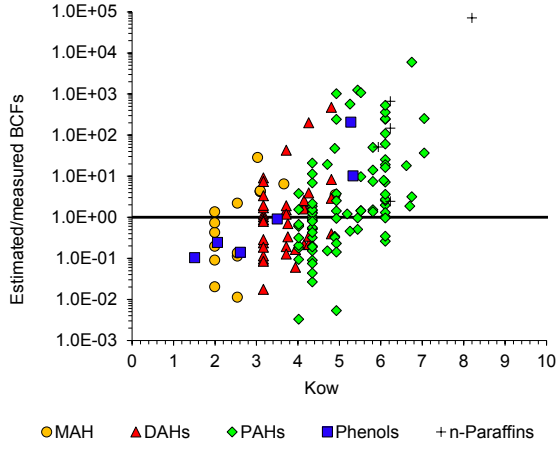


Figure A2 Ratio between estimated and measured bioconcentration factors (BCFs) of aquatic species exposed to oil constituents in relation to the octanol-water partition coefficient Kow of oil constituents. The horizontal line indicates a perfect model fit.

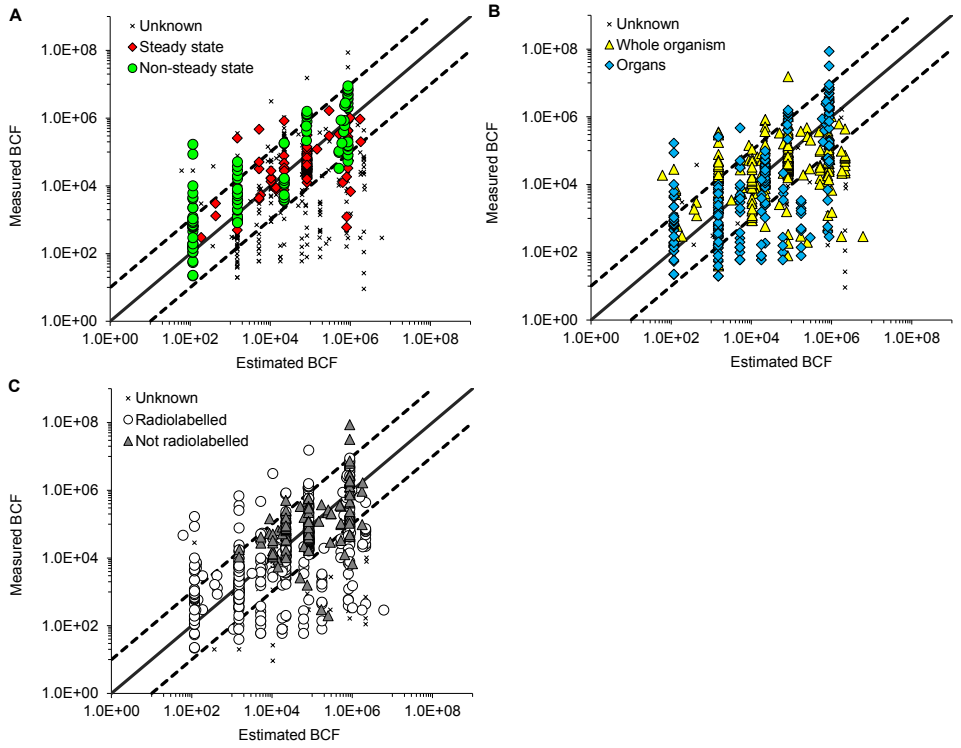


Figure A3 Lipid-normalized BCFs of oil constituents measured in experiments A) at steady or non-steady state, B) in the whole organism or the organism's organs, and C) with radiolabelled or non-radiolabelled compounds versus BCFs estimated with the OMEGA bioaccumulation model for aquatic species from different taxonomic groups. The 1:1 line indicates a perfect model fit. The dashed lines represent a factor of 10 under- and overestimation by OMEGA.

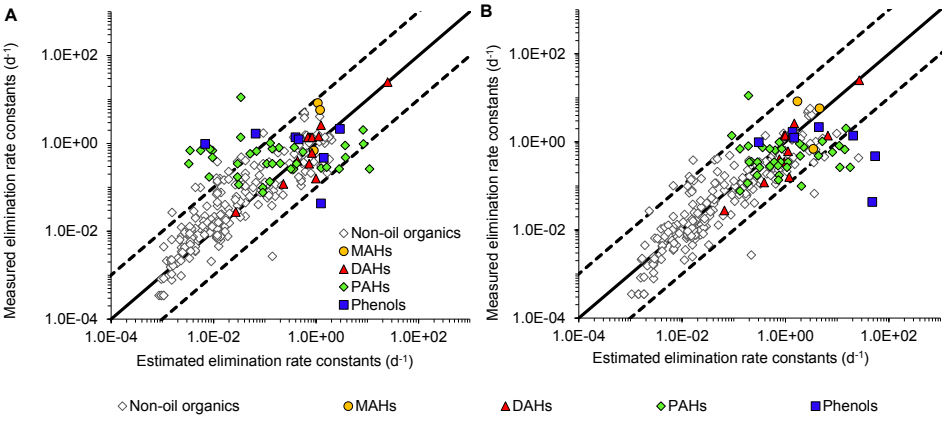


Figure A4 Geometric mean for elimination rate constants of organic constituents from fish measured in experiments versus the estimated geometric mean A) without addition of biotransformation rate constants and B) with addition of biotransformation rate constants to the total elimination rate constant $\Sigma k_{j,out}$. The open diamonds represent persistent organic constituents that have been used previously for model calibration of absorption and elimination rate constants. Coloured dots represent different oil groups. The 1:1 line indicates a perfect model fit. The dashed lines represent a factor 10 under- and overestimation by OMEGA.

Table A3 Number of data (n), coefficients of efficiency (E) and the root-mean-square-errors (RMSE) of log transformed elimination rate constants for fish exposed to oil and non-oil organic constituents estimated without (- $k_{3,out}$) and with (+ $k_{3,out}$) the addition of biotransformation rate constants to the total elimination rate constants ($\Sigma k_{j,out}$)

	Fish elimination – $k_{3,out}$			Fish elimination + $k_{3,out}$ [a]		
Groups	n	E	RMSE	n	E	RMSE
Oil constituents	58	-2.45	1.00	58	-1.25	0.81
Oil groups						
MAHs	3	0.46	0.65	3	0.58	0.57
DAHs	12	0.83	0.30	12	0.67	0.42
PAHs	36	-6.57	1.14	36	-2.21	0.74
Phenols	7	-3.47	1.16	7	-6.20	1.48
Non-oil organic constituents [b]	206	0.73	0.47	206	0.73	0.48

[a] Biotransformation rate constants were gathered from the EPI Suite programme [75].

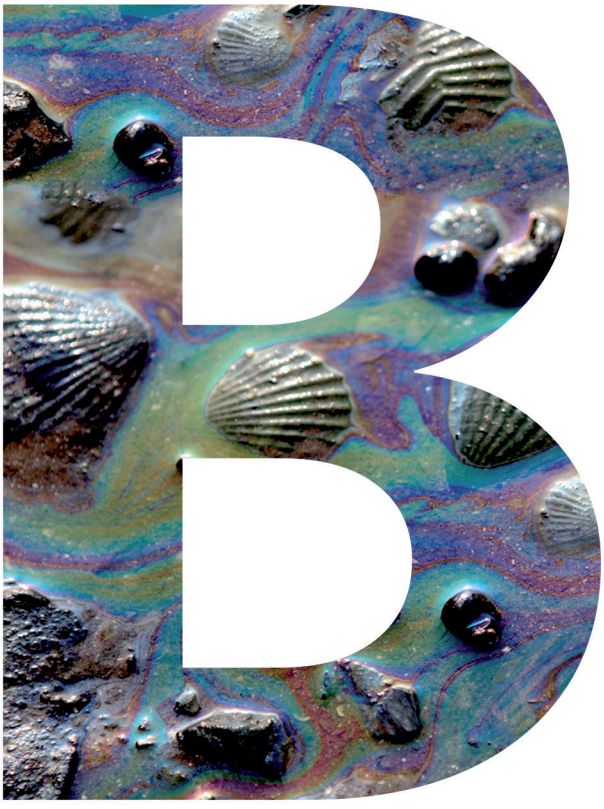
[b] Persistent non-oil organic constituents.

Table A4 Number of data (n), coefficients of efficiency (E) and the root-mean-square-errors (RMSE) of log transformed bioconcentration factors (BCF) for fish exposed to oil and non-oil organic constituents estimated without (- $k_{3,out}$) and with (+ $k_{3,out}$) the addition of biotransformation rate constants to the total elimination rate constants ($\Sigma k_{j,out}$)

	Fish BCF – $k_{3,out}$			Fish BCF + $k_{3,out}$ [a]		
Groups	n	E	RMSE	n	E	RMSE
Oil constituents	61	-2.10	1.43	61	-0.34	1.07
Oil groups						
MAHs	11	0.45	0.72	11	-0.13	0.67
DAHs	16	0.07	0.64	16	0.38	0.63
PAHs	23	-2.03	1.41	23	0.63	0.70
Phenols	6	-3.37	1.19	6	-5.23	1.53
n-Paraffins	5	-9.44	3.40	5	-6.44	2.60
Non-oil organic constituents [b]	74	0.28	1.01	74	0.62	0.73

[a] Biotransformation rate constants were gathered from the EPI Suite programme [75].

[b] Persistent non-oil organic constituents.



B
Appendix to chapter 3

Table B1 The wet weight and lipid fraction of aquatic species [a]

Species scientific name	Common name	Wet weight (kg)	Lipid fraction of wet weight	Reference
Clupea pallasii	Pacific herring	1.2E-07		[293]
			0.050	Default [113]
Average		**1.2E-07**	**0.050**	
Chironomus tentans	Midge	2.9E-06		[294]
		8.8E-06		[295]
		1.1E-05		[295]
		8.3E-06		[295]
		9.3E-06		[295]
		6.9E-06		[295]
		8.3E-06		[295]
		6.3E-06		[295]
		7.7E-06		[295]
		5.9E-06		[295]
		6.7E-06		[295]
		6.3E-06		[295]
		6.4E-06		[295]
			0.010	[27]
Average		**7.3E-06**	**0.010**	
Standard deviation		**2.0E-06**	**-**	
Daphnia magna	Cladoceran	1.4E-06		[6]
		3.4E-07		[44]
		5.5E-07		[296]
			0.030	Default [113]
Average		**6.4E-07**	**0.030**	
Standard deviation		**5.6E-07**	**-**	
Diporeia spp.	Amphipod	5.8E-06		[297]
		3.4E-06		[298]
		4.3E-06		[298]
		3.5E-06		[298]
		2.7E-06		[298]
		3.4E-06		[298]
		3.9E-06		[298]
		2.6E-06		[298]
			0.060	[27]
Average		**3.7E-06**	**0.060**	
Standard deviation		**1.0E-06**	**-**	
Hyalella azteca	Amphipod	4.1E-06		[299]
		4.5E-06		[299]
		3.7E-06		[299]
		3.4E-06		[299]
		8.0E-06		[300]
		2.5E-06		[301]
			0.025	[27]
Average		**4.4E-06**	**0.025**	
Standard deviation		**1.9E-06**	**-**	
Oncorhynchus mykiss	Rainbow trout (juvenile)	2.00E-03		[302]

[a] The average values were used for estimating body burdens with the OMEGA model.

Table B2 The chemicals and species for which we obtained lethal body burdens (LBB) and slope values from the literature

Data type [a]	Chemical	Chemical group [b]	Species	Taxa	Reference
LBB	Fluoro-, chloro-, and bromobenzenes, aniline, naphthalene	Narcotics	Crustaceans, fish	Crustacea, Osteichthyes	[303]
LBB	DDE, PCB	Narcotics	Amphipod	Crustacea	[304]
LBB	Chlorobenzene	Narcotics	Midge	Insecta	[305]
Slope [c]	1,2,4-trichlorobenzene; 1,1,2,2-tetrachloroethane; 1,2 + 1,4-dichlorobenzene	Narcotics	Fathead minnow	Osteichthyes	[306]
Slope [c]	2,2',4,4'-tetrachlorobiphenyl	Narcotics	Amphipod	Crustacea	[307]
Slope [c]	Pentachlorobenzene	Narcotics	Amphipods	Crustacea	[308]
Slope [c]	Halobenzenes	Narcotics	Fish	Osteichthyes	[309]
Slope [d]	Several chemicals	Narcotics	Several aquatic species	Several taxa	[122]
LBB	Naphthalene	DAH	Fathead minnow	Osteichthyes	[128]
LBB	Fluoranthene, Mixture, Naphthalene, Pyrene, Phenanthrene	PAH	Amphipod	Crustacea	[310]
LBB	Fluorene, Pyrene, Phenanthrene	PAH	Amphipod	Crustacea	[26]
LBB	Fluoranthene equivalents (parent + metabolites)	PAH	Amphipods, Midge	Crustacea, Insecta	[308]
LBB	Fluoranthene equivalents (parent + metabolites)	PAH	Amphipods, Fathead minnow, worm	Crustacea, Osteichthyes, Polychaeta	[102]
LBB	Fluoranthene equivalents	PAH	Amphipods, Midge	Crustacea, Insecta	[311]
LBB	Fluoranthene equivalents (parent + metabolites)	PAH	Amphipod	Crustacea	[312]
Slope [c]	Fluorene, Pyrene, Phenanthrene	PAH	Amphipod	Crustacea	[26]
Slope [c]	Fluoranthene	PAH	Amphipod	Crustacea	[312]

[a] LBB = lethal body burden
[b] DAH = dicyclic aromatic hydrocarbon, PAH = polycyclic aromatic hydrocarbon, narcotics = organic chemicals with a narcotic toxicity mode of action (i.e. baseline toxicity) excluding oil constituents
[c] Slope is based on internally measured oil concentrations
[d] Slope is based on externally measured oil concentrations

Table B3 Average, minimum and maximum values and the number of data (n) for the lethal body burden (LBB) and the slope of concentration-response curves

Data classification	LBB (mmol/kg lipid)				Slope (1/β)			
	Average	min-max	*n*	Reference	Average	min-max	*n*	Reference
Narcotics other than oil [a]								
Overall (acute & chronic)	75.4	21.7-136.0	60 [b]	[303-305]	-	-	-	
External concentrations	-	-	-		3.1	0.6-4.8	70	[122]
Internal concentrations	-	-	-		2.7	0.9-24.9	12	[306-309]
Oil constituents								
Overall	63.7	12.3-280	35	[26, 102, 128, 308, 310-312]	4.3	2.4-11.1	4	[26, 312]
Acute (≤ 5 days)	33.8	16.9-107.2	5		-	-	-	
Chronic (> 5 days)	70.9	12.3-280	30		-	-	-	
All data	65.6	12.3-280.0	95		3.0	0.9-24.9	16	

[a] Organic chemicals that exhibit a non-polar narcotic toxicity, e.g. halogenated compounds, alcohols, ethers, ketones.
[b] Based on the mean of measured critical body burdens for 7 different species, including in total 60 data points [303].

Table B4 Experimental conditions for measuring time-varying effects of oil constituents on the survival of aquatic species

Chemical	Species	Phylum	Class	Weight [a]	Lipid [b]	T (d) [c]	Exposure conc. (µg/L) [d]	K_{ow}	Medium [e]	BB [f]	Endpoint	Ref
Fluoranthene	*Chironomus tentans*	Arthropoda	Insecta	7.3E-06	0.01	10	15.6; 31.3; 62.5; 125; 250	$10^{\wedge 5.25}$	FW	Yes	Mortality %	[27]
Benzene	*Clupea pallasii*	Chordata	Actinopterygii	1.2E-07	0.05	2	13.0; 31.9	$10^{\wedge 2.00}$	SW	No	Survival % (larvae)	[114]
Pyrene	*Daphnia magna*	Arthropoda	Branchiopoda	6.4E-07	0.03	21	17.5; 35.0; 70.0	$10^{\wedge 5.18}$	FW	No	Fraction surviving	[29]
Fluoranthene	*Daphnia magna*	Arthropoda	Branchiopoda	6.4E-07	0.03	21	86.2; 172.5	$10^{\wedge 5.25}$	FW	No	Fraction surviving	[29]
Fluoranthene	*Diporeia* spp.	Arthropoda	Malacostraca	3.7E-06	0.06	28	15.6; 31.3; 62.5; 125; 250	$10^{\wedge 5.25}$	FW	Yes	Mortality %	[27]
Fluorene	*Hyalella azteca*	Arthropoda	Malacostraca	4.4E-06	0.03	10	515.3; 698.1; 897.6	$10^{\wedge 4.05}$	FW	No	No. of survivors	[98]
Pyrene	*Hyalella azteca*	Arthropoda	Malacostraca	4.4E-06	0.03	10	70.8; 89.0; 111.2; 139.6	$10^{\wedge 5.18}$	FW	No	No. of survivors	[98]
Fluoranthene	*Hyalella azteca*	Arthropoda	Malacostraca	4.4E-06	0.03	28	15.6; 31.3; 62.5; 125; 250	$10^{\wedge 5.25}$	FW	Yes	Mortality %	[27]
Phenanthrene	*Oncorhynchus mykiss*	Chordata	Actinopterygii	3.5E-03	0.05	22	100	$10^{\wedge 4.65}$	FW/SW	No		[118]
Retene	*Oncorhynchus mykiss*	Chordata	Actinopterygii	3.5E-03	0.05	22	100	$10^{\wedge 6.24}$	FW/SW	No		[118]
Trimethylbenzene	*Pimephales promelas*	Chordata	Actinopterygii	7.0E-04	0.05	4	8089.5	$10^{\wedge 3.42}$	FW	No	Fraction surviving	[97]
Naphthalene	*Pimephales promelas*	Chordata	Actinopterygii	7.0E-04	0.05	4	6049.6; 10304.9	$10^{\wedge 3.35}$	FW	No	Fraction surviving	[97]

[a] Average weight reported in the experimental study or obtained from other studies (Table B1)
[b] Lipid is the lipid fraction of the wet weight, which was either reported in the experimental study or obtained from other studies (Table B1)
[c] T is the experimental duration
[d] All water concentrations are nominal, i.e. not measured after applying the chemical to the water, except for Pimephales promelas and Hyalella azteca exposed to fluorene and pyrene.
[e] FW: fresh water, SW: salt water
[f] Whether there are measured body burdens available in the experimental study.

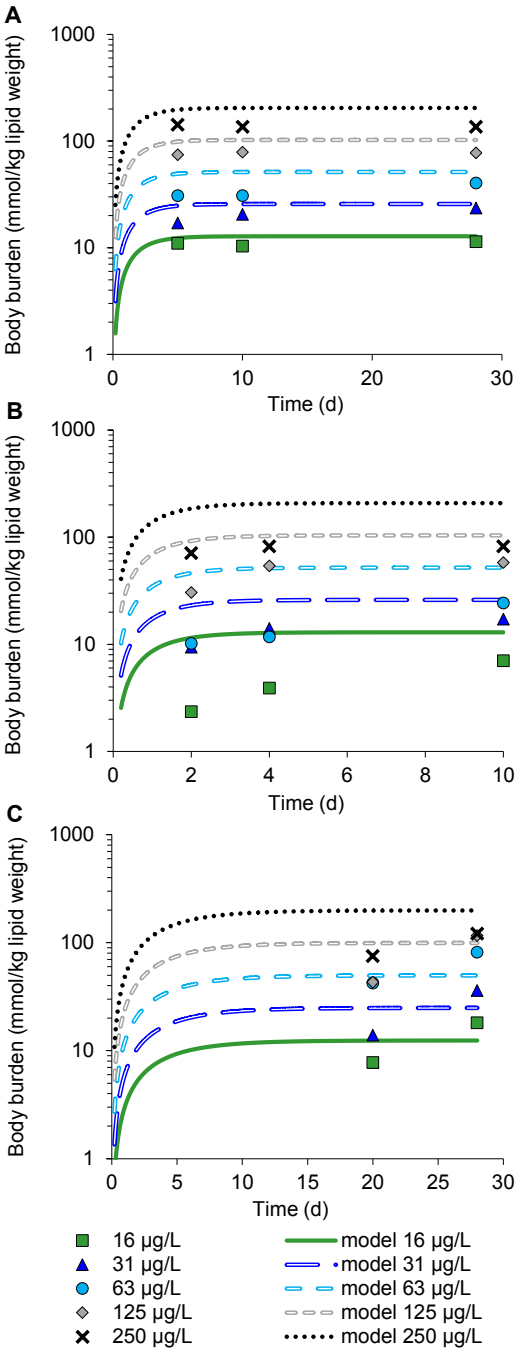


Figure B1 The body burden (mmol/kg lipid weight) measured experimentally (dots) and estimated (lines) with the OMEGA model for the fresh water species *Hyalella azteca* (A), *Chironomus tentans* (B) and *Diporeia* spp. (C) exposed to fluoranthene [308].

Table B5 The RMSE calculated per oil concentration resulting from a 0% change, a factor 2 lower (/ 2) and higher (x 2) lethal body burden (LBB) and slope

Chemical	C_w (µg/L)	Species scientific name	Species common name	n	RMSE no change	RMSE LBB / factor 2	RMSE LBB x factor 2	RMSE slope / factor 2	RMSE slope x factor 2
Fluoranthene	16	*Chironomus tentans*	Midge	3	0.10	0.06	0.10	0.11	0.04
Fluoranthene	31	*Chironomus tentans*	Midge	3	0.21	0.11	0.24	0.25	0.12
Fluoranthene	63	*Chironomus tentans*	Midge	3	0.30	0.32	0.48	0.38	0.27
Fluoranthene	125	*Chironomus tentans*	Midge	3	0.27	0.39	0.37	0.35	0.25
Fluoranthene	250	*Chironomus tentans*	Midge	3	0.07	0.08	0.15	0.08	0.11
Pyrene	18	*Daphnia magna*	Water flea	4	0.15	0.13	0.15	0.15	0.12
Pyrene	35	*Daphnia magna*	Water flea	4	0.04	0.26	0.01	0.00	0.17
Pyrene	70	*Daphnia magna*	Water flea	4	0.20	0.63	0.12	0.11	0.29
Fluoranthene	86	*Daphnia magna*	Water flea	3	0.49	0.84	0.11	0.52	0.48
Fluoranthene	173	*Daphnia magna*	Water flea	3	0.67	0.76	0.36	0.75	0.54
Fluoranthene	16	*Diporeia* spp.	Amphipod	11	0.18	0.14	0.18	0.18	0.12
Fluoranthene	31	*Diporeia* spp.	Amphipod	11	0.24	0.05	0.28	0.28	0.13
Fluoranthene	63	*Diporeia* spp.	Amphipod	15	0.18	0.25	0.37	0.29	0.13
Fluoranthene	125	*Diporeia* spp.	Amphipod	15	0.21	0.31	0.34	0.28	0.18
Fluoranthene	250	*Diporeia* spp.	Amphipod	15	0.28	0.30	0.16	0.30	0.20
Fluoranthene	16	*Hyalella azteca*	Amphipod	11	0.17	0.13	0.17	0.17	0.12
Fluoranthene	31	*Hyalella azteca*	Amphipod	4	0.14	0.13	0.18	0.18	0.08
Fluoranthene	63	*Hyalella azteca*	Amphipod	4	0.14	0.49	0.20	0.13	0.19
Fluoranthene	125	*Hyalella azteca*	Amphipod	15	0.30	0.41	0.37	0.38	0.27
Fluoranthene	250	*Hyalella azteca*	Amphipod	15	0.09	0.10	0.15	0.10	0.11
Fluorene	698	*Hyalella azteca*	Amphipod	4	0.18	0.61	0.14	0.12	0.27
Fluorene	898	*Hyalella azteca*	Amphipod	4	0.30	0.53	0.42	0.29	0.30
Pyrene	89	*Hyalella azteca*	Amphipod	15	0.27	0.63	0.22	0.22	0.31
Pyrene	111	*Hyalella azteca*	Amphipod	15	0.36	0.65	0.28	0.40	0.35
Pyrene	140	*Hyalella azteca*	Amphipod	5	0.38	0.56	0.41	0.44	0.37
Benzene	13000	*Clupea pallasii*	Pacific herring	5	0.12	0.08	0.12	0.12	0.07
Benzene	31900	*Clupea pallasii*	Pacific herring	5	0.36	0.18	0.42	0.42	0.26
Phenanthrene	100	*Oncorhynchus mykiss*	Rainbow trout	11	0.40	0.38	0.41	0.41	0.36
Retene	100	*Oncorhynchus mykiss*	Rainbow trout	11	0.22	0.14	0.23	0.23	0.13
Naphthalene	6050	*Pimephales promelas*	Fathead minnow	4	0.24	0.48	0.27	0.33	0.21
Naphthalene	10305	*Pimephales promelas*	Fathead minnow	4	0.07	0.02	0.39	0.02	0.20
Trimethylbenzene	8090	*Pimephales promelas*	Fathead minnow	4	0.55	0.69	0.18	0.67	0.43
$RMSE_{model}$					0.25	0.34	0.25	0.27	0.22

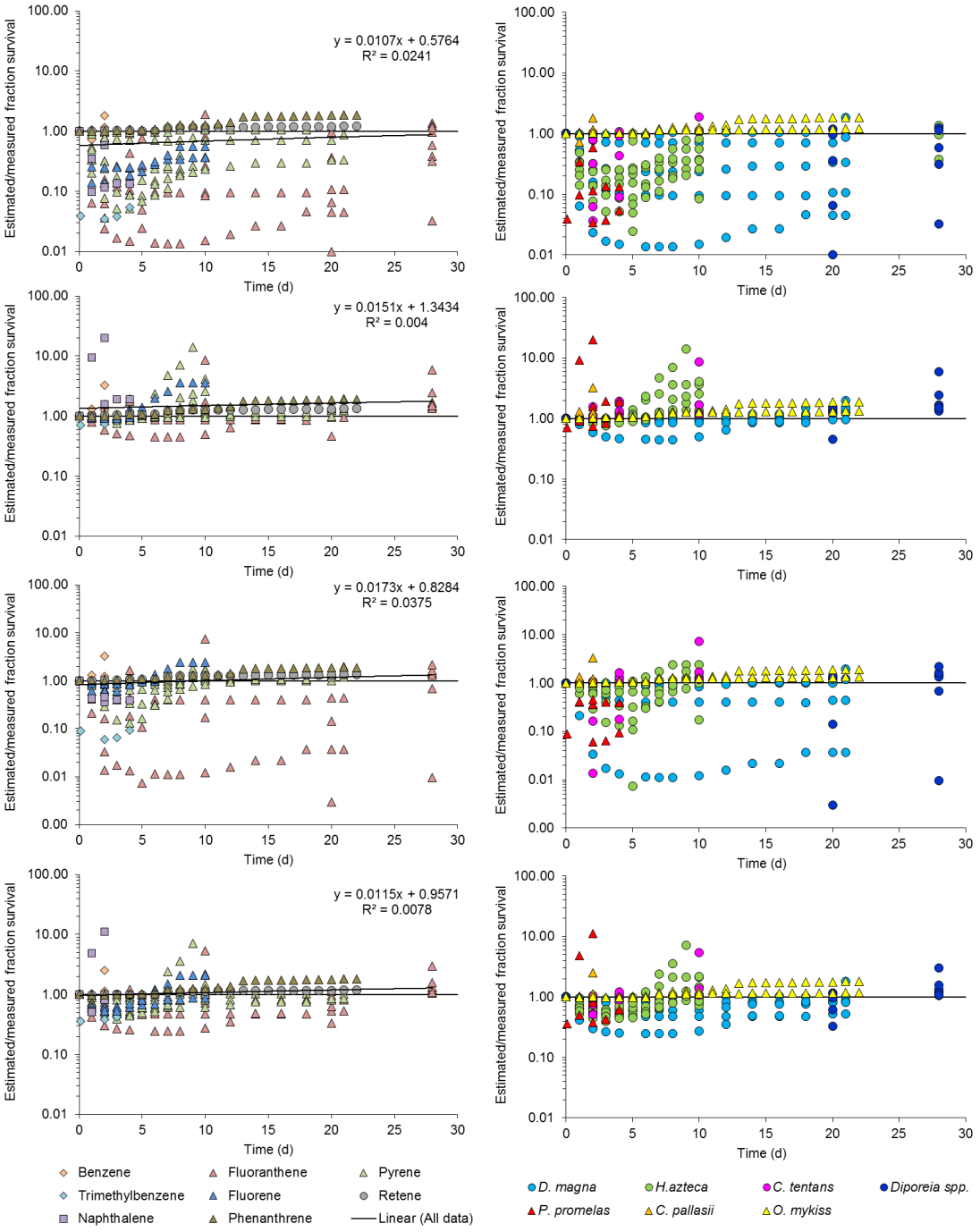


Figure B2. Ratio between estimated and measured fraction survival versus the time (days) for different oil constituents and aquatic species when using A&B) LBB / factor 2, C&D) LBB * factor 2, E&F) slope / factor 2, G&H) slope * factor 2 for crustaceans and fish in the model calculations. Note the different y- axis in figures E & F.

Table B6 The root-mean-square-errors (RMSE) of the log transformed estimated and measured body burdens (BB) of three crustacean species for various concentrations of fluoranthene

Conc. (µg/L)	Species	RMSE	Factor (= $10^{\wedge RMSE}$)
16	*Hyalella azteca*	0.23	1.7
31	*Hyalella azteca*	0.21	1.6
63	*Hyalella azteca*	0.13	1.4
125	*Hyalella azteca*	0.18	1.5
250	*Hyalella azteca*	0.13	1.4
16	*Chironomus tentans*	0.15	1.4
31	*Chironomus tentans*	0.11	1.3
63	*Chironomus tentans*	0.30	2.0
125	*Chironomus tentans*	0.13	1.4
250	*Chironomus tentans*	0.12	1.3
16	*Diporeia* spp.	0.33	2.2
31	*Diporeia* spp.	0.33	2.1
63	*Diporeia* spp.	0.40	2.5
125	*Diporeia* spp.	0.26	1.8
250	*Diporeia* spp.	0.11	1.3
Average		0.21	1.6

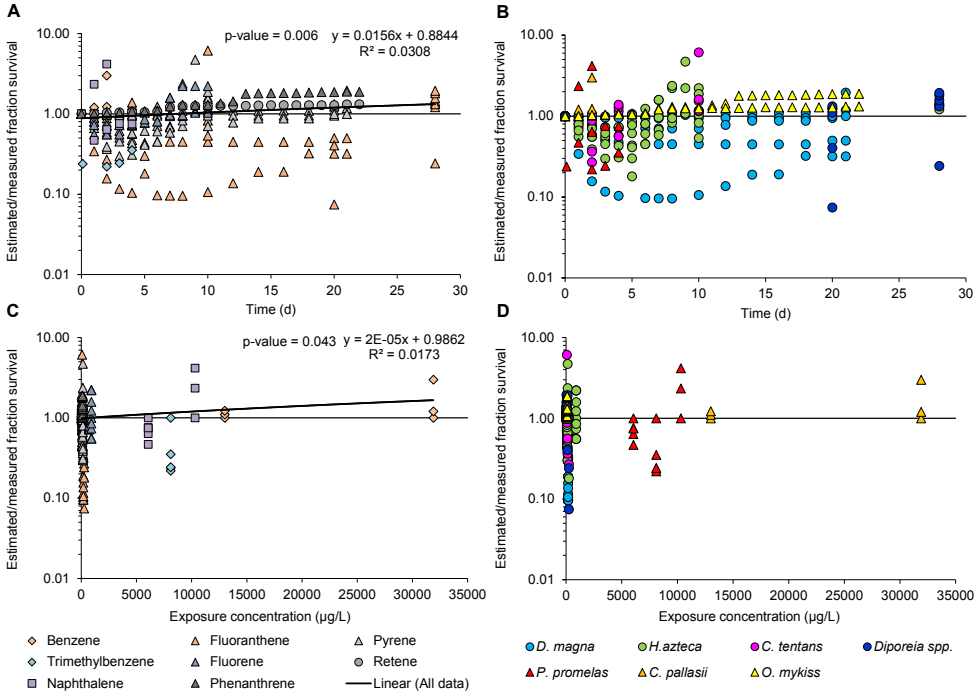


Figure B3 Ratio between estimated and measured fraction survival versus the time (d) (A, B) and exposure concentration (µg/L) (C,D) for different oil constituents (A, C) and aquatic species (B, D).

Table B7 The RMSE calculated per oil concentration resulting from a 0% change, a lower (/ factor 10) and higher (x factor 10) species wet weight and lipid fraction

Chemical	C_w (µg/L)	Species scientific name	Species common name	n	RMSE no change	RMSE wet weight / factor 10	RMSE wet weight x factor 10	RMSE lipid fraction / factor 10	RMSE lipid fraction x factor 10
Fluoranthene	16	*Chironomus tentans*	Midge	3	0.10	0.10	0.10	0.10	0.10
Fluoranthene	31	*Chironomus tentans*	Midge	3	0.21	0.21	0.21	0.20	0.24
Fluoranthene	63	*Chironomus tentans*	Midge	3	0.30	0.30	0.30	0.30	0.47
Fluoranthene	125	*Chironomus tentans*	Midge	3	0.27	0.30	0.20	0.30	0.36
Fluoranthene	250	*Chironomus tentans*	Midge	3	0.07	0.07	0.05	0.07	0.39
Pyrene	18	*Daphnia magna*	Water flea	4	0.15	0.15	0.15	0.15	0.15
Pyrene	35	*Daphnia magna*	Water flea	4	0.04	0.05	0.04	0.05	0.01
Pyrene	70	*Daphnia magna*	Water flea	4	0.20	0.22	0.18	0.24	0.06
Fluoranthene	86	*Daphnia magna*	Water flea	3	0.49	0.51	0.46	0.54	0.17
Fluoranthene	173	*Daphnia magna*	Water flea	3	0.67	0.69	0.63	0.70	0.28
Fluoranthene	16	*Diporeia* spp.	Amphipod	11	0.18	0.18	0.18	0.18	0.18
Fluoranthene	31	*Diporeia* spp.	Amphipod	11	0.24	0.24	0.24	0.23	0.28
Fluoranthene	63	*Diporeia* spp.	Amphipod	15	0.18	0.18	0.19	0.16	0.39
Fluoranthene	125	*Diporeia* spp.	Amphipod	15	0.21	0.21	0.19	0.22	0.39
Fluoranthene	250	*Diporeia* spp.	Amphipod	15	0.28	0.28	0.27	0.28	0.09
Fluoranthene	16	*Hyalella azteca*	Amphipod	11	0.17	0.17	0.17	0.17	0.17
Fluoranthene	31	*Hyalella azteca*	Amphipod	4	0.14	0.14	0.14	0.14	0.17
Fluoranthene	63	*Hyalella azteca*	Amphipod	4	0.14	0.15	0.11	0.15	0.16
Fluoranthene	125	*Hyalella azteca*	Amphipod	15	0.30	0.31	0.27	0.32	0.27
Fluoranthene	250	*Hyalella azteca*	Amphipod	15	0.09	0.09	0.08	0.09	0.25
Fluorene	698	*Hyalella azteca*	Amphipod	4	0.18	0.18	0.18	0.20	0.11
Fluorene	898	*Hyalella azteca*	Amphipod	4	0.30	0.30	0.29	0.30	0.22
Pyrene	89	*Hyalella azteca*	Amphipod	15	0.27	0.31	0.20	0.35	0.23
Pyrene	111	*Hyalella azteca*	Amphipod	15	0.36	0.41	0.28	0.45	0.29
Pyrene	140	*Hyalella azteca*	Amphipod	5	0.38	0.43	0.29	0.47	0.46
Benzene	13000	*Clupea pallasii*	Pacific herring	5	0.12	0.12	0.12	0.08	0.12
Benzene	31900	*Clupea pallasii*	Pacific herring	5	0.36	0.36	0.36	0.33	0.43
Phenanthrene	100	*Oncorhynchus mykiss*	Rainbow trout	11	0.40	0.40	0.40	0.37	0.41
Retene	100	*Oncorhynchus mykiss*	Rainbow trout	11	0.22	0.22	0.22	0.76	0.23
Naphthalene	6050	*Pimephales promelas*	Fathead minnow	4	0.24	0.29	0.14	0.40	0.40
Naphthalene	10305	*Pimephales promelas*	Fathead minnow	4	0.07	0.06	0.12	0.03	0.73
Trimethylbenzene	8090	*Pimephales promelas*	Fathead minnow	4	0.55	0.56	0.50	0.68	0.22
$RMSE_{model}$					0.25	0.25	0.23	0.28	0.26

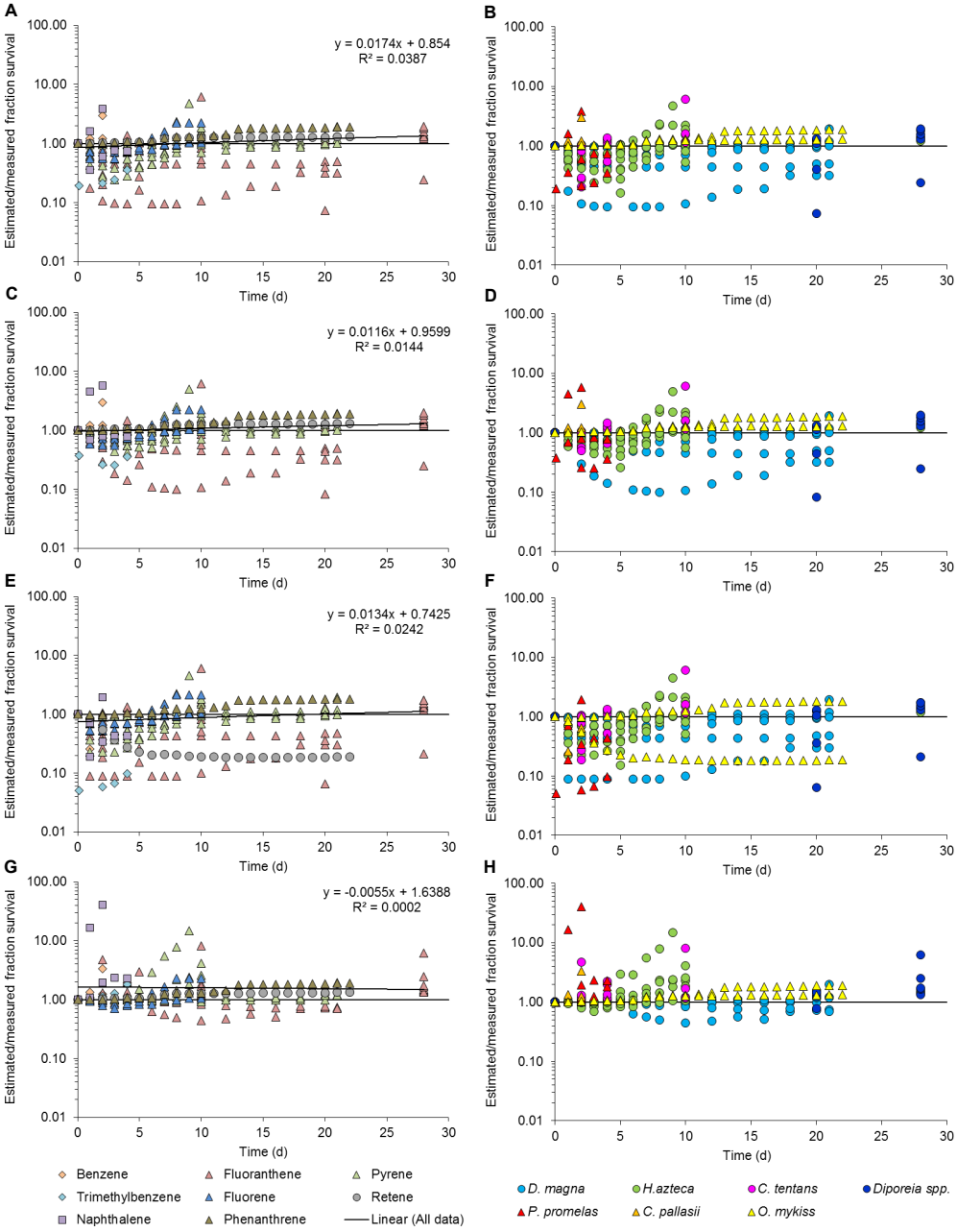


Figure B4. Ratio between estimated and measured fraction survival versus the time (days) for different oil constituents and aquatic species when using A&B) wet weight / factor 10, C&D) wet weight * factor 10, E&F) lipid weight / factor 10, G&H) lipid weight * factor 10 for crustaceans and fish in the model calculations.

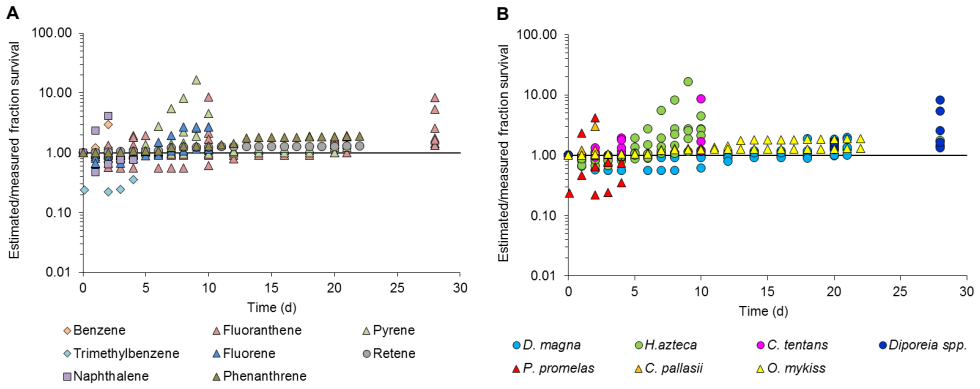


Figure B5 Ratio between estimated and measured fraction survival versus the time (days) for different oil constituents and aquatic species when including a biotransformation rate of 1.15 d^{-1} for crustaceans in the model calculations.

B1. Stochastic death equations

First, the effects of oil constituents on the survival between two consecutive points in time, which is the survival probability $S_{probability}$, of aquatic organisms were calculated relative to the survival representative of a control situation (day^{-1}; Eqn. B1). We assumed the effects to be a logistic function of the estimated body burden [31]:

$$S_{probability} = \frac{1}{1+(\frac{BB_{s,c,t}}{LBB})^{slope}}$$ Equation B1

where $BB_{s,c,t}$ is the time-varying body burden (mmol/kg lipid), LBB the lethal body burden (mmol/kg lipid), i.e. the critical body burden, and the slope represents the inter-individual variation in LBB as represented by the corresponding concentration-response curve [111].

Second, the survival probability per day was scaled to a fraction survival S_{frac} (unitless; Eqn. B2) relative to the survival on t = 0 by

$$S_{frac,t} = S_{prob,t} \times \prod_{i=0}^{t} S_{prob,i}$$ Equation B2

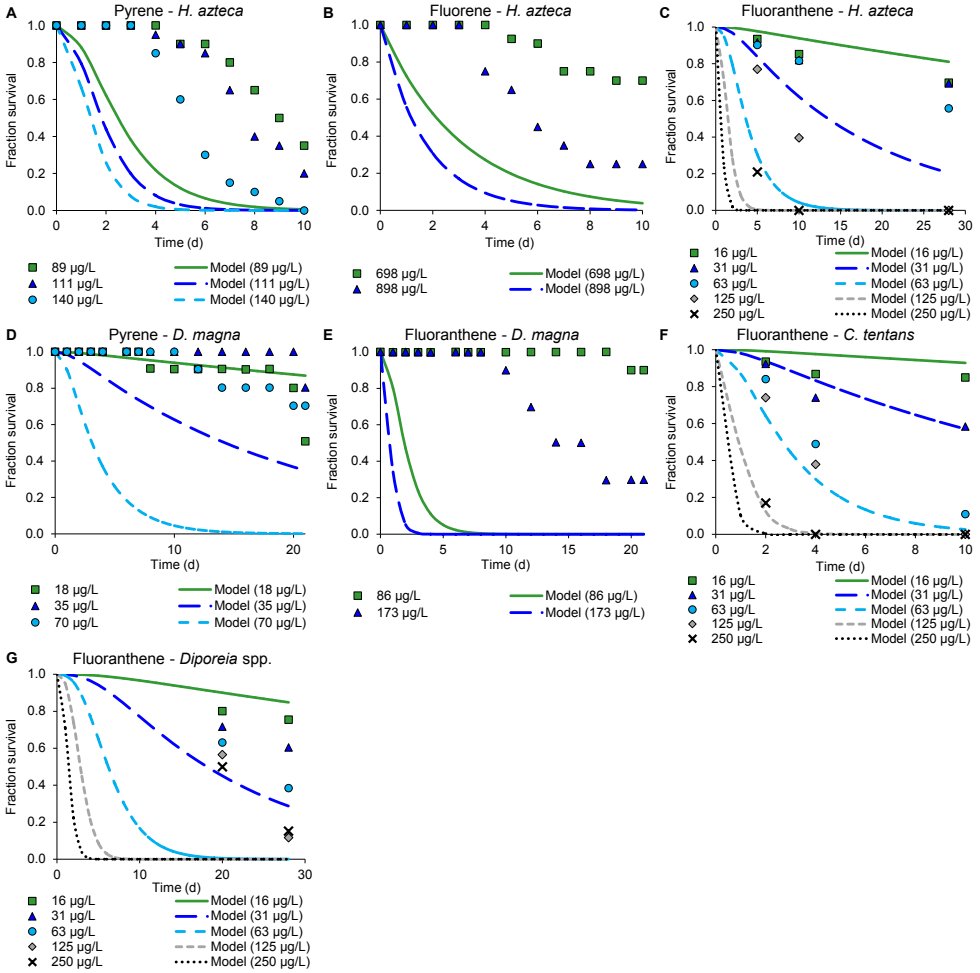


Figure B6 Fraction survival measured experimentally (dots) and estimated with Eqns. B1 and B2 (lines) for the crustaceans Hyalella azteca, *Daphnia magna, Chironomus tentans*, and *Diporeia* spp. and the fish *Pimephales promelas, Clupea pallasii*, and *Oncorhynchus mykiss* exposed to different constant concentrations of oil constituents.

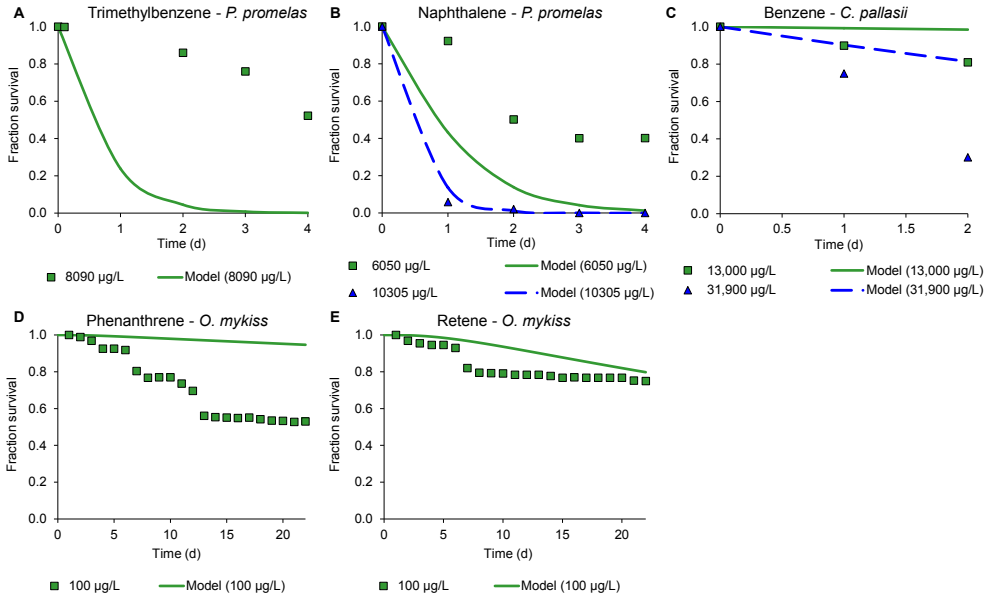
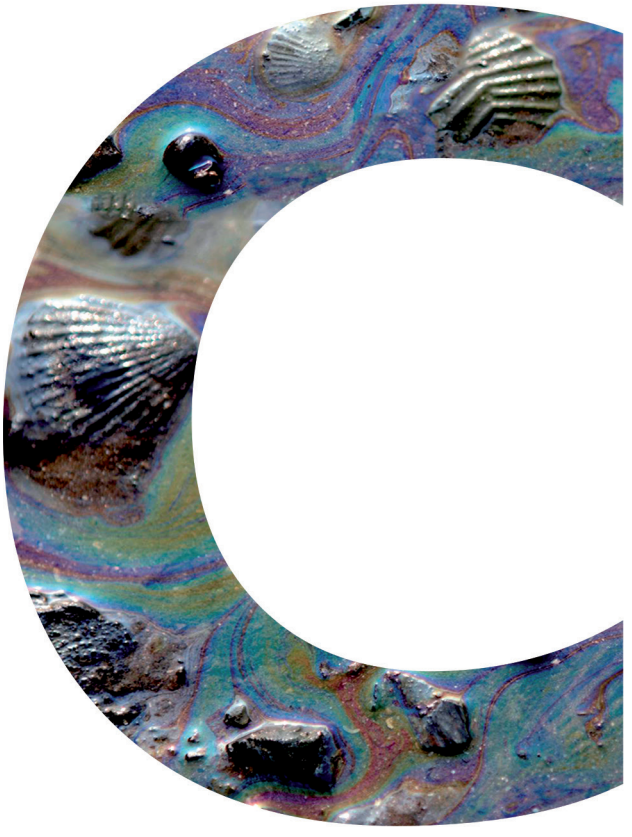


Figure B6 Fraction survival measured experimentally (dots) and estimated with Eqns. B1 and B2 (lines) for the crustaceans Hyalella azteca, *Daphnia magna, Chironomus tentans*, and *Diporeia* spp. and the fish *Pimephales promelas, Clupea pallasii*, and *Oncorhynchus mykiss* exposed to different constant concentrations of oil constituents.



C
Appendix to chapter 4

Table C1 Kow and molecular mass values of the 25 pseudo-components in the OSCAR model [74]

Number	Chemical name	Molecular weight	K_{ow}	log K_{ow}
1	C1-C4 gasses	37.0	11398.2	4.06
2	C5-saturates	66.0	6060.1	3.78
3	C6-saturates	80.5	13263.9	4.12
4	Benzene	78.0	713.8	2.85
5	C7-saturates	99.0	61244.1	4.79
6	C1-Benzene (toluene)	92.0	1772.8	3.25
7	C8-saturates	113.0	168333.4	5.23
8	C2-Benzene (xylenes)	106.0	3877.2	3.59
9	C9-saturates	127.0	535823.9	5.73
10	C3-Benzene	120.0	8750.6	3.94
11	C10-saturates	140.5	991572.1	6.00
12	C4 and C5 Benzenes	141.5	26567.8	4.42
13	C11-C12 (total saturates + aromatics)	156.5	1249750.0	6.10
14	Phenols	130.0	61.7	1.79
15	Naphthalenes 1 (C0-C1-alkylated)	135.0	14988.9	4.18
16	C13-C14 (total saturates + aromatics)	185.5	1571949.0	6.20
17	Naphthalenes 2 (C2-C3-alkylated)	163.0	48435.2	4.69
18	C15-C16 (total saturates + aromatics)	215.5	1978577.0	6.30
19	Polycyclic Aromatic Hydrocarbons 1	177.0	65553.6	4.82
20	C17-C18 (total saturates + aromatics)	238.0	2490916.0	6.40
21	C19-C20 (total saturates + aromatics)	273.0	3122824.0	6.49
22	Unresolved Chromatographic Materials	215.0	4348.7	3.64
23	C21-C25 (total saturates + aromatics)	317.5	3955549.0	6.60
24	Polycyclic Aromatic Hydrocarbons 2	222.5	897059.7	5.95
25	C25+ (total)	465.0	4969529.0	6.70

Table C2 Lethal body burdens (LBB), slopes and lethal-sublethal ratios (qls) of aquatic species exposed to oil constituents [a, b, c]

Parameter	Setting	Chemical	Species	Common name	Value	Unit	Test duration (d)	Endpoint	Ref.
LBB	Average	Naphthalene	*Diporeia* spp.	Amphipod	81.3	mmol/kg lipid	10	50% mortality (LBB)	[310]
LBB	Average	Fluorene	*Hyalella azteca*	Amphipod	37.2	mmol/kg lipid	10	50% mortality (LBB)	[26]
LBB	Average	Fluoranthene	*Hyalella azteca*	Amphipod	55	mmol/kg lipid	10	50% mortality (LBB)	[308]
LBB	Average	Fluoranthene	*Hyalella azteca*	Amphipod	44.3	mmol/kg lipid	10	50% mortality (LBB)	[308]
LBB	Average	Fluoranthene	*Diporeia* spp.	Amphipod	213.5	mmol/kg lipid	10	50% mortality (LBB)	[308]
LBB	Average	Fluoranthene	*Hyalella azteca*	Amphipod	180	mmol/kg lipid	10	50% mortality (LBB)	[102]
LBB	Average	Fluoranthene	*Hyalella azteca*	Amphipod	280	mmol/kg lipid	10	50% mortality (LBB)	[102]
LBB	Average	Fluoranthene	*Hyalella azteca*	Amphipod	66.7	mmol/kg lipid	10	50% mortality (LBB)	[311]
LBB	Average	Phenanthrene	*Diporeia* spp.	Amphipod	136.6	mmol/kg lipid	10	50% mortality (LBB)	[310]
LBB	Average	Phenanthrene	*Hyalella azteca*	Amphipod	51.1	mmol/kg lipid	10	50% mortality (LBB)	[26]
LBB	Average	Pyrene	*Hyalella azteca*	Amphipod	92.2	mmol/kg lipid	10	50% mortality (LBB)	[26]
LBB	Average	Nonylphenol	*Ampelisca abdita*	Amphipod	35	mmol/kg lipid	10	50% mortality (LBB)	[307]
LBB	Worst-case	Fluoranthene	*Chironomus tentans*	Midge	12.3	mmol/kg lipid	10	50% mortality (LBB)	[308]
LBB	Best-case	Fluoranthene	*Diporeia* spp.	Amphipod	200	mmol/kg lipid	28	50% mortality (LBB)	[310]
Slope [d]	Average	Pyrene	*Hyalella azteca*	Amphipod	11.16	mmol/kg	10	Lethality (%)	[26]
Slope [d]	Average	Fluorene	*Hyalella azteca*	Amphipod	4.53	mmol/kg	10	Lethality (%)	[26]
Slope [d]	Average	Phenanthrene	*Hyalella azteca*	Amphipod	4.5	mmol/kg	10	Lethality (%)	[26]
Slope [d]	Average	Fluoranthene	*Hyalella azteca*	Amphipod	3.64	mmol/kg	10	Lethality (%)	[27]
Slope [d]	Worst-case	Pyrene	*Hyalella azteca*	Amphipod	11.16	mmol/kg	10	Lethality (%)	[26]
Slope [d]	Best-case	Fluoranthene	*Hyalella azteca*	Amphipod	2.65	mmol/kg	28	Lethality (%)	[27]
q_{ls}	Average	Benzene	*Ceriodaphnia dubia*	Water flea	1.07	-	7	Acute lethal LC50/Acute sublethal EC50	[313]
q_{ls}	Average	Toluene	*Ceriodaphnia dubia*	Water flea	1.06	-	7	Acute lethal LC50/Acute sublethal EC50	[313]
q_{ls}	Average	Ethylbenzene	*Ceriodaphnia dubia*	Water flea	1.1	-	7	Acute lethal LC50/Acute sublethal EC50	[313]
q_{ls}	Average	m-Xylene	*Ceriodaphnia dubia*	Water flea	1.08	-	7	Acute lethal LC50/Acute sublethal EC50	[313]
q_{ls}	Average	Cyclohexene	*Daphnia magna*	Water flea	1.38	-	15	Chronic lethal LC50/ Chronic sublethal EC50	[314]
q_{ls}	Worst-case	Toluene	*Ceriodaphnia dubia*	Water flea	1.06	-	7	Acute lethal LC50/Acute sublethal EC50	[313]
q_{ls}	Best-case	Cyclohexene	*Daphnia magna*	Water flea	1.38	-	15	Chronic lethal LC50/ Chronic sublethal EC50	[314]

[a] Only data for fresh water species.
[b] In all studies the internal concentration of a chemical is equal to the sum of the parent compound and the corresponding metabolites, except metabolites were not mentioned in [313, 314].
[c] To minimize photodegradation of the chemical and photoinduced chemical toxicity to the organism, ultraviolet filtered light was used in [27, 311]. For the other data we had to assume that the photoinduced toxicity was minimized, as no photoperiod or a method to prevent a possible photoinduced toxicity was mentioned.
[d] The slope is equal to 1 divided by the beta of the concentration-response curve.

Table C3 Variables and parameters for the Rosenzweig-McArthur model estimations of a phytoplankton-zooplankton system of which zooplankton is exposed to crude oil

Symbol [a]	Description	Value	Unit [b]	Calculated from / typical value for	Ref.
Variables					
N_1, N_2	Biomass		$kg \cdot km^{-2}$		c
$k_{m,b}$	Background mortality rate		day^{-1}	$k_{m,b} = k_{p,2}$	c
$k_{m,\ oil}$	Crude oil mortality rate		day^{-1}	$k_{m,\ oil}$ = Eqn. 4.2	d
$k_{m,2}$	Maximum mortality rate of $k_{m,b}$ and $k_{m,\ oil}$		day^{-1}		e
Parameters					
$N_{0,1}$, $N_{0,2}$	Biomass at t = 0	7.78×10^3, 4.57×10^3	$kg \cdot km^{-2}$	$K \approx N = \gamma_N \times m^{\kappa}$	c
β	Slope of Holling type II response for intake of nutrients	1.00	-	Grazers	c
$\gamma_{N,1}$, $\gamma_{N,2}$	Average density coefficient	1.01×10^7, 1.35×10^5	$kg^{\kappa} \cdot km^{-2}$	cold-blooded animals, according to: $\gamma_{N,i} = \frac{F_0 \prod_1^i p_{n,i} p_{a,i} p_{p,i}}{n_i q_T \gamma_p} / (E_s p_{s,i})$	c, f
γ_p	Average production coefficient	7.50×10^{-4}	$kg^{\kappa} \cdot day^{-1}$		c
E_s	Dry matter energy content	2.00×10^4	$kJ \cdot kg^{-1} \cdot dw$		g
$P_{s,1}$, $p_{s,2}$	Dry-to-wet weight	0.10, 0.15			h
$k_{p,1}$, $k_{p,2}$	Average production rate constant [c]	0.98, 0.02	day^{-1}	$k_p = q_t \times \gamma_p \times m^{-\kappa}$	c
$max(k_{n,2})$	Max. consumption rate constant [c]	1.11	day^{-1}	$max(k_{n,2}) = (\ln(R_{0,2})+1)/(p_{an,2} \times p_{pa,2}) \times k_{p,2}$	c
κ	Allometric exponent	0.25	-	densities scale to species mass	c, f
K_1, K_2	Carrying capacity	7.78×10^3, 4.57×10^3	$kg \cdot km^{-2}$	$K = \gamma_N \times m^{\kappa}$	c
m_1	Mass food	3.47×10^{-13}	kg·ind	Geomean for the diatoms *Skeletonema costatum*, *Thalassiosira pseudonana*, *Chaetoceros neogracile*	i, j
m_2	Mass *C. finmarchicus*	1.31×10^{-6}	kg·ind	$m_2 = 10 \times \exp^{(level-1) \times \ln(131/27)/10\hat{}9}$, level = adult stage no. 28	k
$N_{50,1}$	Half saturation density	1.56×10^3	$kg \cdot km^{-2}$	$N_{50} = 0.2 \times K_1$	c
$p_{an,2}$	Fraction assimilated of ingested biomass	0.40	$kg \cdot kg^{-1}$	Herbivores	c
$p_{pa,2}$	Fraction produced of assimilated biomass	0.25	$kg \cdot kg^{-1}$	Invertebrates	c
q_t	Temperature correction	1.00	-		c
r_1	Maximum intrinsic rate of increase	0.6	day^{-1}	Average of 0.6-0.8 and 0.4-0.6 for cultured diatoms from the Barents Sea	l, m
$R_{0,1}$, $R_{0,2}$	Potential lifetime fecundity	2, 55	#·ind	Unicellular organisms	c, f
⊠ t	Time between two measurements	0.04	day		
Δ					

[a] 1 = diatoms, 2 = *Calanus finmarchicus* / zooplankton.
[b] Kg is in wet weight. The unit $kg \cdot km^{-2}$ was transformed to $gC \cdot m^{-2}$ by dividing the simulated biomass N^1 and N^2 by 10,000.
[c] [15], [d] [31], [e] [144], [f] [190], [g] [82], [h] [11], [i] [68], [j] [315], [k] [263], [l] [316], [m] [143]

Table C4 Contribution of 25 pseudo-components (%) to the total crude oil concentration in the water at the start (day 0) and end (day 50) of a hypothetical oil spill in OSCAR for the years 1995, 1997 and 2001

Number	Chemical name	1995		1997		2001	
		Day 0 (%)	Day 50 (%)	Day 0 (%)	Day 50 (%)	Day 0 (%)	Day 50 (%)
1	C1-C4 gasses	5.7	1.7	8.6	9.9	11.2	2.4
2	C5-saturates	5.0	1.5	7.5	8.5	10.1	2.2
3	C6-saturates	1.3	0.6	2.7	8.9	5.8	0.6
4	Benzene	0.6	0.4	1.9	0.5	3.2	0.3
5	C7-saturates	0.2	0.1	0.6	9.7	0.7	0.1
6	C1-Benzene (toluene)	8.0	5.5	16.7	4.9	11.9	3.0
7	C8-saturates	7.2	11.1	0.5	16.4	0.3	1.7
8	C2-Benzene (xylenes)	24.7	22.4	21.0	10.3	9.3	12.9
9	C9-saturates	1.6	26.0	0.1	9.6	0.0	3.7
10	C3-Benzene	24.5	15.6	7.0	7.8	2.8	24.3
11	C10-saturates	0.0	0.0	0.0	0.1	0.0	0.0
12	C4 and C5 Benzenes	0.5	0.2	0.0	0.2	0.0	0.8
13	C11-C12 (total saturates + aromatics)	0.0	0.0	0.0	0.0	0.0	0.0
14	Phenols	13.1	6.4	32.6	7.6	44.5	13.2
15	Naphthalenes 1 (C0-C1-alkylated)	4.3	2.2	0.5	1.2	0.2	10.6
16	C13-C14 (total saturates + aromatics)	0.0	0.0	0.0	0.0	0.0	0.0
17	Naphthalenes 2 (C2-C3-alkylated)	2.4	2.7	0.2	1.7	0.1	12.1
18	C15-C16 (total saturates + aromatics)	0.0	0.0	0.0	0.0	0.0	0.0
19	Polycyclic Aromatic Hydrocarbons 1	0.9	1.7	0.1	1.0	0.0	6.8
20	C17-C18 (total saturates + aromatics)	0.0	0.0	0.0	0.0	0.0	0.0
21	C19-C20 (total saturates + aromatics)	0.0	0.0	0.0	0.0	0.0	0.0
22	Unresolved Chromatographic Materials	0.0	0.0	0.0	0.0	0.0	0.0
23	C21-C25 (total saturates + aromatics)	0.0	0.0	0.0	0.0	0.0	0.0
24	Polycyclic Aromatic Hydrocarbons 2	0.0	1.8	0.0	1.7	0.0	5.4
25	C25+ (total)	0.0	0.0	0.0	0.0	0.0	0.0

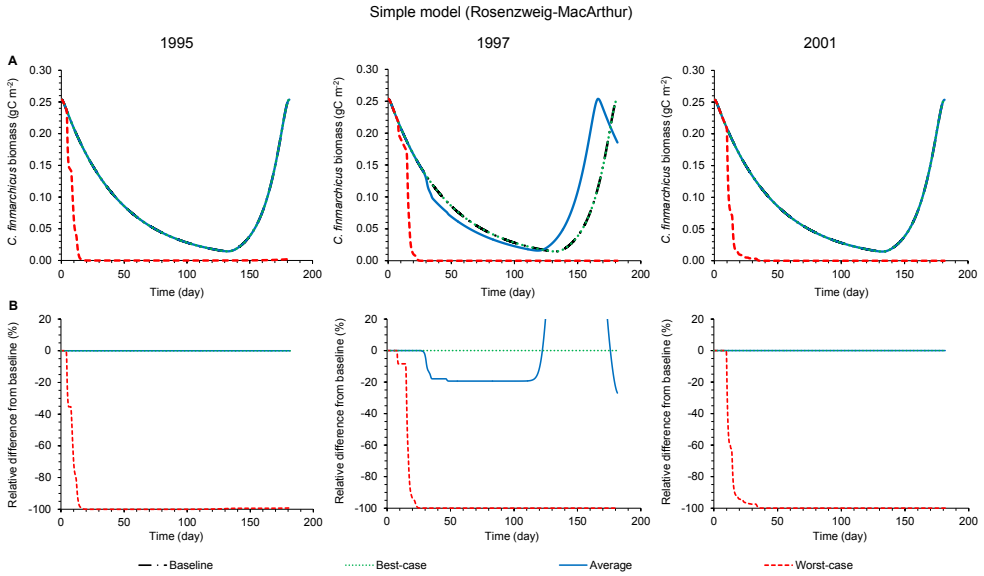


Figure C1 Using TBBs similar to the ones calculated by the complex SINMOD model to determine: A) Average C. finmarchicus biomass (gC·m^{-2}) and B) time series of the relative difference (%) in C. finmarchicus biomass between the baseline and three oil toxicity parameter settings at a grid cell close to the "Nordland VI – point 2" release point of crude oil in the years 1995, 1997 and 2001 for the Rosenzweig-MacArthur consumer-resource model. The best-case, average and worst-case ecotoxicological parameter values cause low, average and high effects of crude oil on the organism and the baseline represents simulation data without oil exposure. The lines for the baseline, average and best-case parameter settings are overlapping.

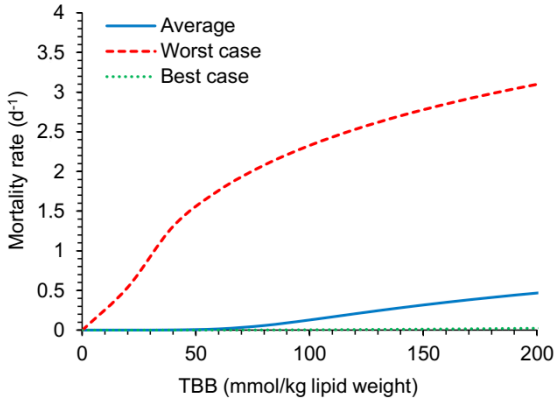


Figure C2 Mortality rates (d^{-1}) of crude oil exposure to *C. finmarchicus* estimated by using Eqn. 4.2 for different total body burdens (TBBs; mmol/kg lipid weight).

D
Appendix to chapter 5

D1. Fatty acid extraction

Hydrolysis of total lipids of diatoms and methylation to fatty acid methyl esters (FAMEs) for FA analysis was achieved by a modified one-step derivatisation method after Abdulkadir and Tsuchiya [317]. The boron trifluoride-methanol reagent was replaced by a 2.5% H_2SO_4-methanol solution since BF3-methanol can cause artefacts or loss of PUFAs [318]. The fatty acid Methylnonadecanoate C19:0 (Fluka 74208) was added as an internal standard for the quantification. Samples were centrifuged (Eppendorf centrifuge 5810R) and vacuum dried (Rapid Vap LABCONCO). The FAMEs obtained from the extracts of the diatoms in each of the triplicated atrazine treatments were analysed using a Hewlet Packard 6890N GC coupled to a mass spectrometer (HP 5973). All samples were run in splitless mode, with a 1 µl injection per run, at an injector temperature of 250 °C, using a HP88 column (60m×25mm i.d., Df=0.20; Agilent J&W; Agilent Co., USA) with He flow rate of 1.3 ml/min. The oven temperature was programmed at 50°C for 2 min, followed by a ramp at 25°C min to 75 °C, then a second ramp at 2°C min to 230 °C with a final 4 min hold.

FAMEs were identified by comparison with the retention times and mass spectra of authentic standards and available ion spectra in WILEY mass spectral libraries, and analysed with the software MSD ChemStation (Agilent Technologies). Quantification of individual FAMEs was accomplished by the use of external standards (SupelcoTM 37 Component FAME Mix, Supelco # 47885, Sigma-Aldrich Inc., USA). The quantification function of each individual FAME was obtained by linear regression applied to the chromatographic peak areas and corresponding known concentrations of the standards (ranging from 5 to 150 µg/ml).

Shorthand FA notations of the form A:BωX were used, where A represents the number of carbon atoms, B gives the number of double bonds and X gives the position of the double bond closest to the terminal methyl group [319].

D2. Generalized additive mixed models

D2.1 Methods

Two generalized additive mixed models (gamm) were constructed to test if the measured diatom density and Fv/Fm significantly changed with time and atrazine concentration [320]. One gamm had diatom density and one gamm had Fv/Fm as a response variable (y). Both models had time and log10(atrazine concentration + 1) as co-variates. We suspected interactions between time and atrazine concentrations; therefore we included the two co-variates in one smoother f. The expected value of the response Y was therefore given as [321]:

$$g^{-1}(E(y|time, atrazine)) = intercept + f(time, atrazine) \qquad \text{Equation D1}$$

where *g* is a link function, i.e. a function that relates the predictors to the response and f is a thin plate spline function expressing the combined effect of atrazine and time. The response variable was assumed to follow a Gaussian distribution and the link function was the identity function, i.e. g(x) = x. The model residuals (i.e. differences between modelled and observed values) were assumed to be normally distributed with mean 0 and variance σ^2 [321]. Because the data are time series, we included an autocorrelation structure to account for correlation between the residuals of two different points in time (t and s) [321]:

$$\mathrm{cor}(\varepsilon_s,\varepsilon_t) = 1 \text{ if } t = s \text{ and } \mathrm{cor}(\varepsilon_s,\varepsilon_t) = \rho^{|t-s|} \text{ else} \qquad \text{Equation D2}$$

D2.2 Results

The residuals (i.e. differences between modelled and observed values) of the statistical regression models used were normally distributed and did not show any pattern with time or atrazine concentration (Figure a). This implies homogeneity and normality of the model residuals and thus that the assumptions underlying the regression are valid and the obtained *p*-values can be judged as reliable. The statistical regression models explained > 96% of the variation in diatom densities and > 65% in the maximum quantum yield of photosynthetic activity (Fv/Fm). The fitted smooth functions f(time, atrazine) for diatom density and Fv/Fm are given on the next page (Figure b).

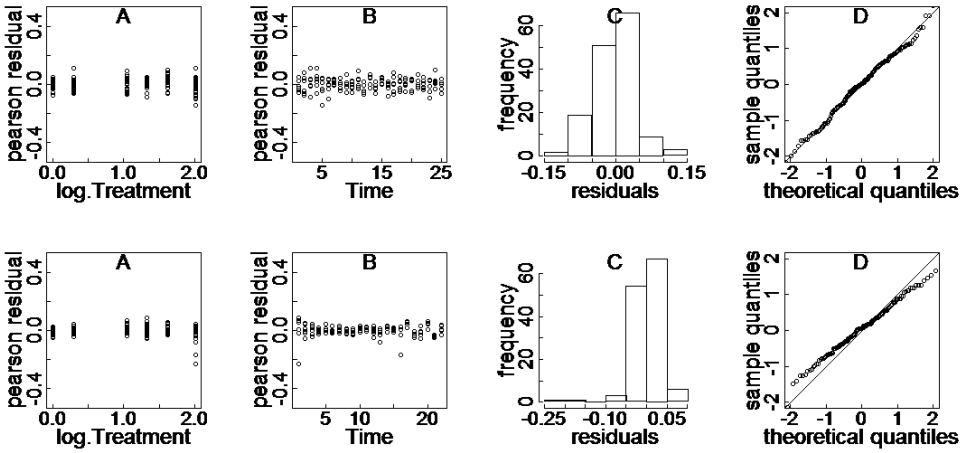


Figure a Patterns of model residuals of diatom density (above) and quality (Fv/Fm; below) with co-variates atrazine (A) and time (B) and the distribution of the residuals in a histogram and QQ-plot.

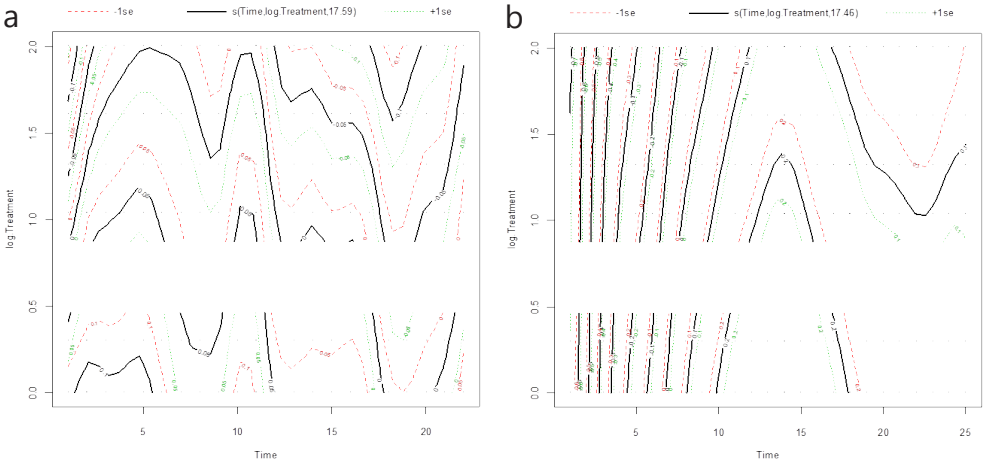


Figure b The fitted smooth function f(time, atrazine). The black lines are isoboles and connect combinations of time and atrazine treatment that elicit the same effect on a) diatom density and b) the maximum quantum yield of photosynthetic activity (Fv/Fm). The effect sizes are given as Arabic numbers with these isoboles. The coloured lines are the standard errors around the isoboles.

Table D1 Diatom and copepod body weights (kg) determined for Rosenzweig-McArthur model simulations of a zoo-phytobenthos system

Species	Wet weight (kg)	Calculated from	References
Diatom	1.62E-12	Geometric mean of 5 diatom species:	
		Chaetoceros neogracile (43.1 pg/cell)	
		Navicula minima (8340 pg/cell)	[68, 315,
		Skeletonema costatum (52.2 pg/cell)	322]
		Thalassiosira pseudonana (19.8 pg/cell)	
		Thalassiosira weissflogii (300 pg/cell)	
Copepod	1.53E-08	Dry weight (µg) = 13.95 × body length (~ 0.6 mm) - 5.32	[323-325]
		Wet weight = dry weight × 5	[82]

Table D2 Nominal and measured atrazine concentrations in µg/L (average of three replicas per treatment) in the experiment with only diatoms and the grazing experiment with both diatoms and copepods

Nominal	Start diatom experiment	End diatom experiment [a]	Start grazing experiment	Before replacing solutions in grazing experiment [b]	End grazing experiment [c]
0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
1	1.0	0.8			
10	9.7	8.7			
20	18.0	17.0	21.0	18.3	< 1.0
40	42.0	41.0			
100	123.0	99.0	118.0	108.7	1.9

[a] Measured after 4 weeks.
[b] Measured after 5 days.
[c] Measured 4 days after the solution with atrazine and stable isotopes was replaced by artificial seawater (salinity 32).

Table D3 Specific uptake of ^{13}C labelled *S. robusta* diatoms ($\Delta\delta^{13}C$) grown under different atrazine concentrations (µg/l) by an individual copepod (*D. palustris*)

Treatment	Replica	$\delta^{13}C$ (µg)	$\Delta\delta^{13}C$ (µg) [a]
Field control [b, c]	A	-17.1	
	B	-15.0	
	C	-17.0	
Atrazine concentration			
0	A	493.4	25.5
	B	1032.5	52.4
	C	274.7	14.6
20	A	256.1	13.6
	B	304.1	15.3
	C	309.1	17.1
100	A	178.5	9.7
	B	62.7	4.0
	C	36.1	2.6

[a] $\Delta\delta^{13}C$ = ($\delta^{13}C$sample - average $\delta^{13}C_{field\ control}$) divided by the number of analysed copepods per replica.
[b] The field control represents the amount of ^{13}C that was present in the copepods before being fed with atrazine exposed diatoms.
[c] The average $\delta^{13}C$ of replica A, B and C was -16.4 µg.

Table D4 Total uptake of ^{13}C labelled *S. robusta* diatoms grown under different atrazine concentrations (µg/l) by the copepod *D. palustris* (µg ^{13}C/µg C copepod) after 4 days

Atrazine treatment	Replica	Total uptake
0	A	0.034
	B	0.070
	C	0.020
20	A	0.025
	B	0.029
	C	0.029
100	A	0.047
	B	0.019
	C	0.013

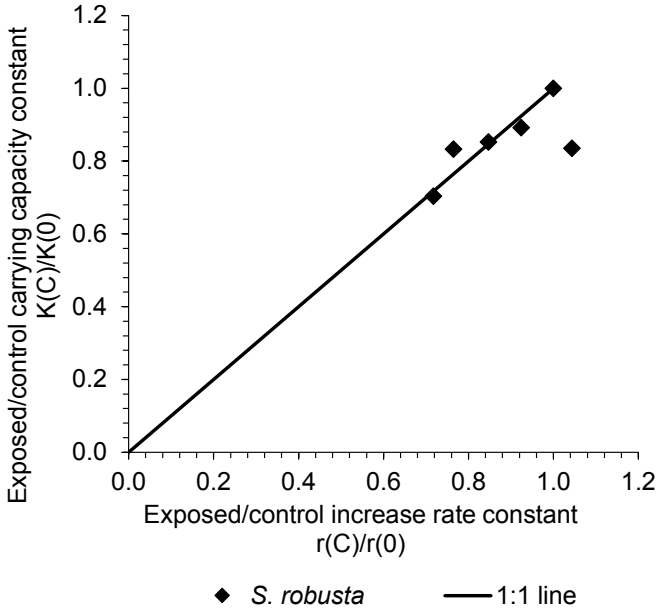


Figure D1 Ratios of control and atrazine exposed population carrying capacity K(C)/K(0) versus ratios of control and atrazine exposed population intrinsic rate of increase r(C)/r(0) of *S. robusta* (dots) determined by fitting the logistic growth model (Eqn. 5.2 [31]) to the observed diatom densities and a 1:1 line to confirm the relationship between r(C)/r(0) and K(C)/K(0).

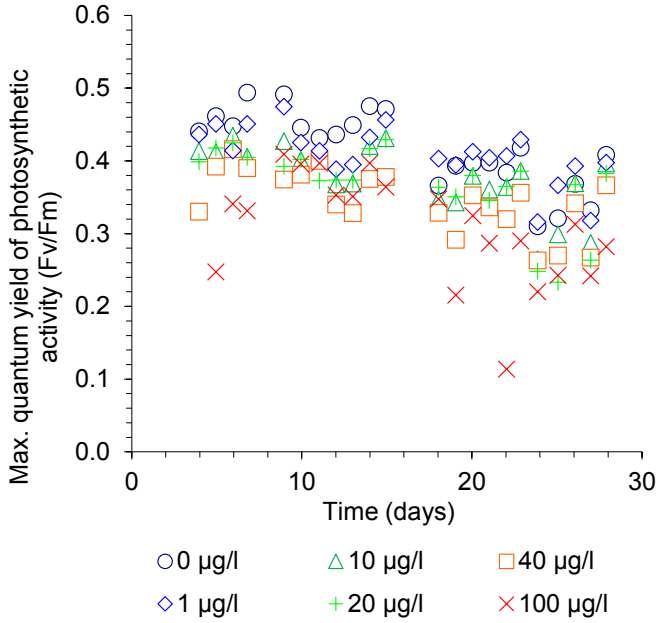


Figure D2 Average maximum quantum yield of photosynthetic activity (Fv/Fm) as a measure of diatom quality versus time for a control (0 µg/l) and five atrazine (1, 10, 20, 40 and 100 µg/l) treatments.

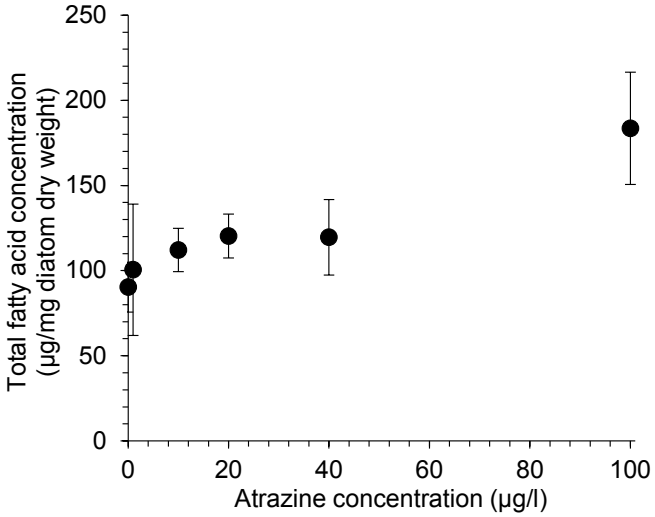


Figure D3 Average total fatty acid concentration (µg) per mg dry weight of the diatom *S. robusta* and standard deviations (error bars) for a control (0 µg/l) and five atrazine (1, 10, 20, 40 and 100 µg/l) exposure treatments.

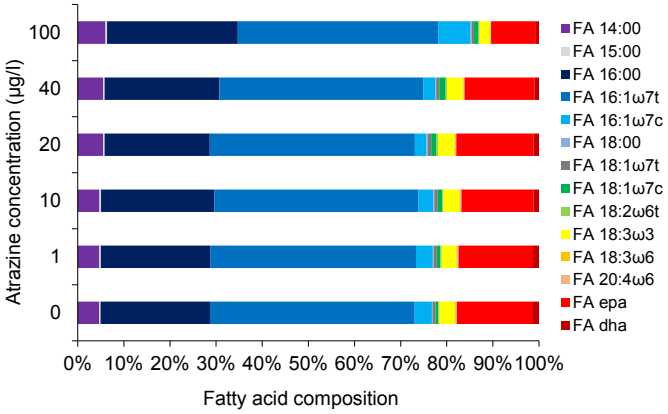


Figure D4 Fatty acid composition in the diatom *S. robusta* after control (0 µg/l) and five atrazine (1, 10, 20, 40 and 100 µg/l) exposure treatments for 28 days.

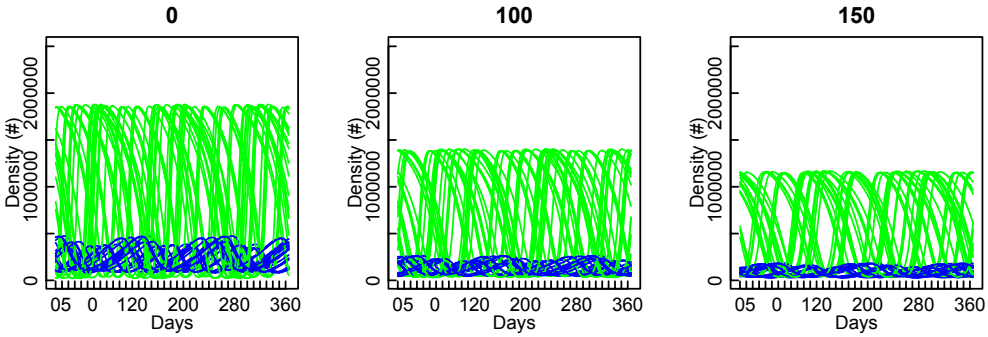
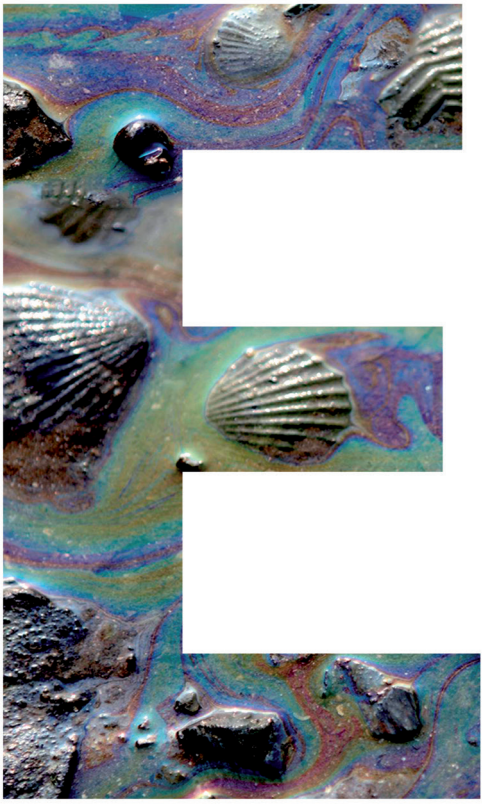


Figure D5 Predicted oscillations of population densities of phytobenthos (green) and zoobenthos (blue) at 20 different random values of the allometric coefficients. The sensitivity analysis included 100 model outputs; only 20 are shown for clarity.



E
Appendix to chapter 6

E1. Comparison between measured HC_{50} and calculated LC_{50} concentrations in relation to a narcotic toxic mode of action.

Organic chemicals like oil are expected to exhibit a narcotic toxicity mode of action [116, 117]. The critical concentration (CBB; critical body burden) needed to produce a lethal effect is highly dependent on the response concentration in the water (LC_{50}), the hydrophobicity of the toxicant (log K_{ow}), and the lipid content in an organism [113, 121]. LC_{50} response concentrations were calculated for the individual oil components naphthalene and 2-methyl-naphthalene to examine whether effect concentrations collected from literature and databases corresponded well with effect concentrations expected from a narcotic toxicity mode of action. The estimation of the LC_{50} (µg/l) is defined by [116]:

$$LC_{50} = \frac{CBB}{BCF} \qquad \text{Equation E1}$$

Where BCF is the bioconcentration factor on a lipid basis. For organic compounds the bioconcentration factor is usually expressed on a lipid basis where BCF and K_{ow} (octanol-water partition coefficient) have approximately the same value.[326] The CBB is approximately constant for different chemicals that act by means of narcosis, ranging from 0.04 to 0.16 mol/kg lipid [2, 5, 121, 246]. Hence, in Eqn. E1, for CBB the average of 0.10 mol/kg lipid was used, together with the molar mass of the toxicant (g/mol) to calculate the toxicant concentration in lipid. One kilogram and one liter of lipid were presumed to be equal. Estimated LC_{50} values were compared with the average toxicity (HC_{50}; hazardous concentration for 50% of the organisms) of temperate and polar species groups for naphthalene and 2-methyl-naphthalene (Table 6.2) [327]. For crude oil, no LC_{50} values could be derived from the narcotic body burden because octanol-water partition coefficients (K_{ow}) differ for each oil fraction.

The estimated LC_{50} values for naphthalene and 2-methyl-naphthalene were respectively $5.7 \cdot 10^3$ µg/l and $1.4 \cdot 10^3$ µg/l. Measured HC_{50} values for naphthalene, 2-methyl-naphthalene and crude oil, which consists partly of these components, were in the same order of magnitude (see Table 6.2). The effect concentrations collected from literature and database were thus in the range expected from nonpolar narcosis.

Table E1 Polar marine toxicity data

Toxicant [a]	Species common name	Species scientific name	Phylum	Class	Endpoint [b]	Life stage	Temp. (°C) [c]	Duration (days)	Conc. (µg/l) [d]	Reference
2-Methyl-naphthalene	Sea snail	*Acmae tessulata*	Mollusca	Gastropoda	LC50			4	350	[206]
2-Methyl-naphthalene	Amphipod	*Anonyx nugax*	Arthropoda	Malacostraca	LC50		4.3-7.7	4	1920	[206]
2-Methyl-naphthalene	Arctic cod	*Boreogadus saida*	Chordata	Actinopterygii	LC50		4.3-7.7	4	800	[206]
2-Methyl-naphthalene	Clam	*Chlamys islandica*	Mollusca	Bivalva	LC50		4.3-7.7	4	2750	[206]
2-Methyl-naphthalene	Atlantic cod	*Gadus morhua*	Chordata	Actinopterygii	LC50			2	2900	[328]
2-Methyl-naphthalene	Atlantic cod	*Gadus morhua*	Chordata	Actinopterygii	LC50			2	3080	[329]
2-Methyl-naphthalene	Atlantic cod	*Gadus morhua*	Chordata	Actinopterygii	LC50			2	3300	[329]
2-Methyl-naphthalene	Atlantic cod	*Gadus morhua*	Chordata	Actinopterygii	LC50			4	2000	[328]
2-Methyl-naphthalene	Shrimp	*Gammarus sp.*	Arthropoda	Malacostraca	LC50		4.3-7.7	4	1340	[206]
2-Methyl-naphthalene	American plaice	*Hippoglossoides platessoides*	Chordata	Actinopterygii	NR-LETH			4	3000	[330]
2-Methyl-naphthalene	Common periwinkle	*Littorina littorea*	Mollusca	Gastropoda	LC50		4.3-7.7	4	1230	[206]
2-Methyl-naphthalene	Sea snail	*Margarites helicina*	Mollusca	Gastropoda	LC50		4.3-7.7	4	5010	[206]
2-Methyl-naphthalene	Sea spider	*Nymphon gracile*	Arthropoda	Pycnogonida	LC50		4.3-7.7	4	5420	[206]
2-Methyl-naphthalene	Pink shrimp	*Pandalus borealis*	Arthropoda	Malacostraca	LC50		4.3-7.7	4	1560	[206]
2-Methyl-naphthalene	Shrimp	*Sclerocragnon boreas*	Arthropoda	Malacostraca	LC50		4.3-7.7	4	1700	[206]
2-Methyl-naphthalene	Green sea urchin	*Strongylocentrotus droebachiensis*	Echinodermata	Echinoidea	NR-LETH			1	3300	[329]
2-Methyl-naphthalene	Green sea urchin	*Strongylocentrotus droebachiensis*	Echinodermata	Echinoidea	NR-LETH			2	2900	[328]
2-Methyl-naphthalene	Green sea urchin	*Strongylocentrotus droebachiensis*	Echinodermata	Echinoidea	NR-LETH			2	3000	[328]
2-Methyl-naphthalene	Green sea urchin	*Strongylocentrotus droebachiensis*	Echinodermata	Echinoidea	NR-LETH			2	3080	[329]
2-Methyl-naphthalene	Green sea urchin	*Strongylocentrotus droebachiensis*	Echinodermata	Echinoidea	LC50		4.3-7.7	4	680	[206]
Naphthalene	Amphipod	*Anonyx nugax*	Arthropoda	Malacostraca	LC50		4.3-5.3	8	2000	[331]
Naphthalene	Amphipod	*Anonyx nugax*	Arthropoda	Malacostraca	LC50		6.5-7.3	8	1200	[331]
Naphthalene	Amphipod	*Anonyx nugax*	Arthropoda	Malacostraca	LC50		9.3-9.9	8	1500	[331]
Naphthalene	Amphipod	*Boeckosimus nanseni*	Arthropoda	Malacostraca	LC50		4.3-5.3	8	5300	[331]
Naphthalene	Amphipod	*Boeckosimus nanseni*	Arthropoda	Malacostraca	LC50		6.5-7.3	8	2500	[331]
Naphthalene	Amphipod	*Boeckosimus nanseni*	Arthropoda	Malacostraca	LC50		9.3-9.9	8	2300	[331]
Naphthalene	Arctic cod	*Boreogadus saida*	Chordata	Actinopterygii	LC50		5.3-7.5	8	1500	[331]
Naphthalene	Arctic cod	*Boreogadus saida*	Chordata	Actinopterygii	LC50		1.5-2.5	8	1200	[331]
Naphthalene	Arctic cod	*Boreogadus saida*	Chordata	Actinopterygii	LC50		7.2-9.8	8	1500	[331]
Naphthalene	Arctic cod	*Boreogadus saida*	Chordata	Actinopterygii	LC50		1.3-1.7	8	1200	[331]
Naphthalene	Shortscale eualid	*Eualus suckleyi*	Arthropoda	Malacostraca	LC50		6-6.9	4	1390	[332]
Naphthalene	Atlantic cod	*Gadus morhua*	Chordata	Actinopterygii	LC50	Eggs		4	5300	[329]
Naphthalene	Amphipod	*Gammaracanthus loricatus*	Arthropoda	Malacostraca	LC50		1.5-2.5	8	2100	[331]
Naphthalene	Alaska fourhorn sculpin	*Oncocottus hexacornis*	Chordata	Actinopterygii	LC50		1.3-1.7	8	1000	[331]

Toxicant [a]	Species common name	Species scientific name	Phylum	Class	Endpoint [b]	Life stage	Temp. (°C) [c]	Duration (days)	Conc. (µg/l) [d]	Reference
Naphthalene	Alaska fourhorn sculpin	*Oncocottus hexacornis*	Chordata	Actinopterygii	LC50		1.5-2.5	8	1100	[331]
Naphthalene	Alaska fourhorn sculpin	*Oncocottus hexacornis*	Chordata	Actinopterygii	LC50		5.3-7.5	8	1600	[331]
Naphthalene	Alaska fourhorn sculpin	*Oncocottus hexacornis*	Chordata	Actinopterygii	LC50		7.2-9.8	8	1700	[331]
Naphthalene	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	LC50			1	920	[333]
Naphthalene	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	LC50			2	1010	[332]
Naphthalene	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	LC50			2	890	[332]
Naphthalene	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	LC50		10.2-11.6	2	900	[332]
Naphthalene	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	LC50		10.2-11.6	2	960	[332]
Naphthalene	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	LC50			2	990	[332]
Naphthalene	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	LC50		8	4	1200	[334]
Naphthalene	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	LC50		12	4	1240	[244]
Naphthalene	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	LC50		4	4	1370	[244]
Naphthalene	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	LC50		8	4	1840	[244]
Naphthalene	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	LC50		12	1	1380	[244]
Naphthalene	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	LC50		4	1	1560	[244]
Naphthalene	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	LC50		8	1	1840	[244]
Naphthalene	Humpy shrimp	*Pandalus goniurus*	Arthropoda	Malacostraca	LC50		12	4	971	[244]
Naphthalene	Humpy shrimp	*Pandalus goniurus*	Arthropoda	Malacostraca	LC50		8	4	1020	[244]
Naphthalene	Humpy shrimp	*Pandalus goniurus*	Arthropoda	Malacostraca	LC50		4	4	2160	[244]
Naphthalene	Humpy shrimp	*Pandalus goniurus*	Arthropoda	Malacostraca	LC50		12	1	1290	[244]
Naphthalene	Humpy shrimp	*Pandalus goniurus*	Arthropoda	Malacostraca	LC50		8	1	2060	[244]
Naphthalene	Humpy shrimp	*Pandalus goniurus*	Arthropoda	Malacostraca	LC50		4	1	2210	[244]
Naphthalene	Green sea urchin	*Strongylocentrotus droebachiensis*	Echinodermata	Echinoidea	NR-LETH			2	2700	[328]
Naphthalene	Green sea urchin	*Strongylocentrotus droebachiensis*	Echinodermata	Echinoidea	NR-LETH			2	3070	[329]
Naphthalene	Green sea urchin	*Strongylocentrotus droebachiensis*	Echinodermata	Echinoidea	NR-LETH			4	2780	[328]
Prudhoe Bay crude oil	Amphipod	*Anonyx nugax*	Arthropoda	Malacostraca	LC50		4.5-6.5	4	32000	[335]
Prudhoe Bay crude oil	Amphipod	*Anonyx nugax*	Arthropoda	Malacostraca	LC50			4	38000	[336]
Cook Inlet crude oil	Amphipod	*Anonyx nugax*	Arthropoda	Malacostraca	LC50		3.2-3.8	8	2300	[331]
Cook Inlet crude oil	Tube-snout	*Aulorhynchus flavidus*	Chordata	Actinopterygii	LC50			4	2550	[227]
Cook Inlet crude oil	Tube-snout	*Aulorhynchus flavidus*	Chordata	Actinopterygii	TLm	Adult	4-12	4	1155	[337]
Prudhoe Bay crude Oil	Amphipod	*Boeckosimus edwardsi*	Arthropoda	Malacostraca	LC50			4	44000	[336]
Cook Inlet crude oil	Arctic cod	*Boreogadus saida*	Chordata	Actinopterygii	LC50		1.5-2.5	8	1600	[331]
Prudhoe Bay crude oil	Calanoid copepod	*Calanus hyperboreus*	Arthropoda	Maxillopoda	LC50			4	73000	[336]
Cook Inlet crude oil	Pink scallop	*Chlamys hericus*	Mollusca	Bivalvia	LC50			4	3940	[227]

Toxicant [a]	Species common name	Species scientific name	Phylum	Class	Endpoint [b]	Life stage	Temp. (°C) [c]	Duration (days)	Conc. (µg/l) [d]	Reference
Cook Inlet crude oil	Pink scallop	*Chlamys hericus*	Mollusca	Bivalvia	LC50			4	2000	[231]
Prudhoe Bay crude oil	Pacific pink scallop	*Chlamys rubida*	Mollusca	Bivalvia	TLm	Adult	3.7-10.2	4	1697	[338]
No. 2 fuel oil	Pink scallop	*Chlamys rubida*	Mollusca	Bivalvia	TLm		3.7-10.2	4	712	[338]
Prudhoe Bay crude oil	Grass shrimp	*Crangon alaskensis*	Arthropoda	Malacostraca	LC50			4	870	[227]
Cook Inlet crude oil	Saffron cod	*Eleginus gracilis*	Chordata	Actinopterygii	TLm	Adult	4-12	1	2138	[337]
Cook Inlet crude oil	Saffron cod	*Eleginus gracilis*	Chordata	Actinopterygii	TLm	Adult	4-12	4	1965	[337]
No. 2 fuel oil	Saffron cod	*Eleginus gracilis*	Chordata	Actinopterygii	TLm	Adult	4-12	4	2608	[337]
Prudhoe Bay crude oil	Kelp shrimp	*Eualus suckleyi*	Chordata	Actinopterygii	LC50			4	1860	[227]
Prudhoe Bay crude oil	Amphipod	*Gammarus setosus*	Arthropoda	Malacostraca	LC50		41395	4	53000	[335]
Prudhoe Bay crude oil	Amphipod	*Gammarus setosus*	Arthropoda	Malacostraca	LC50		3.8-5.2	4	56000	[335]
Cook Inlet crude oil	Great sculpin	*Myoxocephalus polyacanthocephalus*	Chordata	Actinopterygii	LC50			4	3960	[227]
Cook Inlet crude oil	Pelagic mysid	*Mysis relicta*	Chordata	Actinopterygii	LC50		4.4	4	2700	[331]
Norman Wells crude oil	Pelagic mysid	*Mysis oculata*	Arthropoda	Malacostraca	LC50			4	4510	[228]
Norman Wells crude oil	Pelagic mysid	*Mysis oculata*	Arthropoda	Malacostraca	LC50			4	486	[228]
Norman Wells crude oil	Pelagic mysid	*Mysis oculata*	Arthropoda	Malacostraca	LC50			4	624	[228]
Norman Wells crude oil	Pelagic mysid	*Mysis oculata*	Arthropoda	Malacostraca	LC50			4	7570	[228]
Prudhoe bay crude oil	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	TLm	Adult	3.7-10.2	4	1156	[338]
Cook Inlet crude oil	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	TLm	Adult	4-12	1	3560	[337]
Cook Inlet crude oil	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	TLm	Adult	4-12	4	2517	[337]
No. 2 fuel oil	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	TLm	Adult	3.7-10.2	4	721	[338]
No. 2 fuel oil	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	TLm	Adult	4-12	1	792	[337]
No. 2 fuel oil	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	TLm	Adult	4-12	4	721	[337]
Cook Inlet crude oil	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	LC50			4	1690	[227]
Cook Inlet crude oil	Pink salmon	*Oncorhynchus gorbuscha*	Chordata	Actinopterygii	LC50		7-10	5	1200	[231]
Prudhoe Bay crude oil	Amphipod	*Onisimus litoralis*	Arthropoda	Malacostraca	LC50			4	47000	[335]
Prudhoe Bay crude oil	Amphipod	*Onisimus litoralis*	Arthropoda	Malacostraca	LC50			4	49000	[336]
Prudhoe Bay crude oil	Amphipod	*Onisimus litoralis*	Arthropoda	Malacostraca	LC50			4	68000	[335]
Cook Inlet crude oil	Hairy hermit crab	*Pagurus hirsutinsculus*	Arthropoda	Malacostraca	TLm	Adult	4-12	4	2672	[337]
Prudhoe Bay crude oil	Pink shrimp	*Pandalus borealis*	Arthropoda	Malacostraca	TLm	Adult	3.7-10.2	4	1730	[338]
Cook Inlet crude oil	Pink shrimp	*Pandalus borealis*	Arthropoda	Malacostraca	TLm	Adult	4-12	1	2491	[337]
Cook Inlet crude oil	Pink shrimp	*Pandalus borealis*	Arthropoda	Malacostraca	TLm	Adult	4-12	4	2095	[337]
No. 2 fuel oil	Pink shrimp	*Pandalus borealis*	Arthropoda	Malacostraca	TLm	Adult	4-12	1	338	[337]
No. 2 fuel oil	Pink shrimp	*Pandalus borealis*	Arthropoda	Malacostraca	TLm	Adult	4-12	4	187	[337]
Cook Inlet crude oil	Pink shrimp	*Pandalus borealis*	Arthropoda	Malacostraca	LC50			4	4940	[227]

Toxicant [a]	Species common name	Species scientific name	Phylum	Class	Endpoint [b]	Life stage	Temp. (°C) [c]	Duration (days)	Conc. (µg/l) [d]	Reference
Cook Inlet crude oil	Humpy shrimp	*Pandalus goniurus*	Arthropoda	Malacostraca	LC50			4	1790	[227]
Cook Inlet crude oil	Humpy shrimp	*Pandalus goniurus*	Arthropoda	Malacostraca	TLm	Adult	4-12	1	1991	[337]
Cook Inlet crude oil	Humpy shrimp	*Pandalus goniurus*	Arthropoda	Malacostraca	TLm	Adult	4-12	4	1707	[337]
Prudhoe Bay crude oil	Humpy shrimp	*Pandalus goniurus*	Arthropoda	Malacostraca	TLm	Adult	3.7-10.2	4	1033	[338]
No. 2 fuel oil	Humpy shrimp	*Pandalus goniurus*	Arthropoda	Malacostraca	TLm	Adult	4-12	4	1504	[337]
No. 2 fuel oil	Humpy shrimp	*Pandalus goniurus*	Arthropoda	Malacostraca	TLm	Adult	3.7-10.2	4	1504	[338]
Prudhoe Bay crude oil	King crab	*Paralithodes camtschatica*	Arthropoda	Malacostraca	TLm	Adult	3.7-10.2	4	1927	[338]
Cook Inlet crude oil	King crab	*Paralithodes camtschatica*	Arthropoda	Malacostraca	TLm	Adult	4-12	1	4448	[337]
Cook Inlet crude oil	King crab	*Paralithodes camtschatica*	Arthropoda	Malacostraca	TLm	Adult	4-12	4	3629	[337]
No. 2 fuel oil	King crab	*Paralithodes camtschatica*	Arthropoda	Malacostraca	TLm	Adult	3.7-10.2	4	4539	[338]
No. 2 fuel oil	King crab	*Paralithodes camtschatica*	Arthropoda	Malacostraca	TLm	Adult	4-12	4	4539	[337]
Cook Inlet crude oil	King crab	*Paralithodes camtschatica*	Arthropoda	Malacostraca	LC50			4	3690	[227]
Cook Inlet crude oil	King crab	*Paralithodes camtschatica*	Arthropoda	Malacostraca	LC50		7-10	4	1400	[231]
Cook Inlet crude oil	Dolly varden	*Salvelinus malma*	Chordata	Actinopterygii	LC50			4	1550	[227]
Prudhoe bay crude oil	Dolly varden	*Salvelinus malma*	Chordata	Actinopterygii	TLm	Adult	3.7-10.2	4	902	[338]
Cook Inlet crude oil	Dolly varden	*Salvelinus malma*	Chordata	Actinopterygii	TLm	Adult	4-12	1	2802	[337]
Cook Inlet crude oil	Dolly varden	*Salvelinus malma*	Chordata	Actinopterygii	TLm	Adult	4-12	4	2534	[337]
No. 2 fuel oil	Dolly varden	*Salvelinus malma*	Chordata	Actinopterygii	TLm	Adult	3.7-10.2	4	2038	[338]
No. 2 fuel oil	Dolly varden	*Salvelinus malma*	Chordata	Actinopterygii	TLm	Adult	4-12	4	2038	[337]
Cook Inlet crude oil	Walleye pollock	*Theragra chalcogrammus*	Chordata	Actinopterygii	LC50			4	1730	[227]

[a] Toxicity values for oil resulted from experiments with water-soluble fractions (WSF).
[b] TL_m values were converted from ppm (µl/l) to mg/l with the densities of No. 2 fuel oil (0.89 mg/µl), Prudhoe bay (0.82 mg/µl), and Cook Inlet (0.86 mg/µl) crude oil.
[c] Temperature (˚C)
[d] Experimental concentration (µg/l)

Table E2 Temperate marine toxicity data

Toxicant [a]	Species common name	Species scientific name	Phylum	Class	Endpoint [b]	Life stage	Temp. (°C) [c]	Duration (days)	Conc. (µg/l) [d]	Reference
2-Methyl-naphthalene	Dungeness crab	*Cancer magister*	Arthropoda	Malacostraca	LC50		13	4	1300	[339]
2-Methyl-naphthalene	Dungeness crab	*Cancer magister*	Arthropoda	Malacostraca	LC50		13	2	5000	[339]
2-Methyl-naphthalene	Sheepshead minnow	*Cyprinodon variegatus*	Chordata	Actinopterygii	LC50			1	2000	[340]
2-Methyl-naphthalene	European seabass	*Dicentrarchus labrax*	Chordata	Actinopterygii	LC50		11-15	4	3060	[206]
2-Methyl-naphthalene	Copepod	*Eurytemora affinis*	Arthropoda	Maxillopoda	LC50		15	1	1499	[341]
2-Methyl-naphthalene	Shrimp	*Gammarus sp.*	Arthropoda	Malacostraca	LC50		11-15	4	550	[206]
2-Methyl-naphthalene	Sea snail	*Gibbula umbilicalis*	Mollusca	Gastropoda	LC50		11-15	4	1910	[206]
2-Methyl-naphthalene	Blue mussel	*Mytilus edulis*	Mollusca	Bivalvia	LC50		11-15	4	8130	[206]
2-Methyl-naphthalene	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	LC50			1	1700	[340]
2-Methyl-naphthalene	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	LC50		20	1	1650	[342]
2-Methyl-naphthalene	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	LC50		20	2	1400	[342]
2-Methyl-naphthalene	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	LC50		20	4	1100	[342]
2-Methyl-naphthalene	Sea snail	*Patella depressa*	Mollusca	Gastropoda	LC50		11-15	4	4450	[206]
2-Methyl-naphthalene	Brown shrimp	*Penaeus aztecus*	Arthropoda	Malacostraca	LC50		21	4	600	[343]
2-Methyl-naphthalene	Brown shrimp	*Penaeus aztecus*	Arthropoda	Malacostraca	LC50			1	700	[340]
2-Methyl-naphthalene	European flounder	*Platichthys flesus*	Chordata	Actinopterygii	LC50			4	6000	[330]
2-Methyl-naphthalene	Acorn barnacle	*Semibalanus balanoides*	Arhtropoda	Maxillopoda	LC50		11-15	4	4800	[206]
Naphthalene	Blue crab	*Callinectes sapidus*	Arthropoda	Malacostraca	LC50	Adult		1	1980	[232]
Naphthalene	Blue crab	*Callinectes sapidus*	Arthropoda	Malacostraca	LC50	Adult		1	2250	[232]
Naphthalene	Blue crab	*Callinectes sapidus*	Arthropoda	Malacostraca	LC50	Adult		1	3120	[232]
Naphthalene	Dungeness crab	*Cancer magister*	Arthropoda	Malacostraca	LC50			4	2000	[339]
Naphthalene	Pacific oyster	*Crassostrea gigas*	Mollusca	Bivalvia	LC50	Eggs	20-21.5	2	110000	[344]
Naphthalene	Vase tunicate	*Ciona intestinalis*	(Uro)Chordata	Ascidiacea	LC50	Eggs-larvae		2	4280	[230]
Naphthalene	Sheepshead minnow	*Cyprinodon variegatus*	Chordata	Actinopterygii	LC50	OS		1	2400	[340]
Naphthalene	Scud	*Elasmopus pectinicrus*	Arthropoda	Malacostraca	LC50		23	4	2680	[345]
Naphthalene	Scud	*Elasmopus pectinicrus*	Arthropoda	Malacostraca	LC50		23	1	3650	[345]
Naphthalene	Scud	*Elasmopus pectinicrus*	Arthropoda	Malacostraca	LC50		23	2	2800	[345]
Naphthalene	Copepod	*Eurytemora affinis*	Arthropoda	Maxillopoda	LC50		15	1	3798	[341]
Naphthalene	Copepod	*Eurytemora affinis*	Arthropoda	Maxillopoda	NR-LETH			1	1000	[346]
Naphthalene	Mummichog	*Fundulus heteroclitus*	Chordata	Actinopterygii	LC50			4	5300	[347]
Naphthalene	Purple shore crab	*Hemigrapsus nudus*	Arthropoda	Malacostraca	LC50			8	1100	[348]
Naphthalene	Purple shore crab	*Hemigrapsus nudus*	Arthropoda	Malacostraca	LC50			8	2100	[348]
Naphthalene	Purple shore crab	*Hemigrapsus nudus*	Arthropoda	Malacostraca	LC50			8	2800	[348]
Naphthalene	Fertile venus	*Katelysia opima*	Mollusca	Bivalvia	LC50			4	57000	[349]

Toxicant [a]	Species common name	Species scientific name	Phylum	Class	Endpoint [b]	Life stage	Temp. (°C) [c]	Duration (days)	Conc. (µg/l) [d]	Reference
Naphthalene	Fertile venus	*Katelysia opima*	Mollusca	Bivalvia	LC50			1	74000	[349]
Naphthalene	Fertile venus	*Katelysia opima*	Mollusca	Bivalvia	LC50			2	68000	[349]
Naphthalene	Fertile venus	*Katelysia opima*	Mollusca	Bivalvia	LC50			3	64000	[349]
Naphthalene	Blue mussel	*Mytilus edulis*	Mollusca	Bivalvia	LC50	Egg-D-veliger		2	9920	[230]
Naphthalene	Opossum shrimp	*Neomysis americana*	Arthropoda	Malacostraca	LC50		15	4	1280	[350]
Naphthalene	Opossum shrimp	*Neomysis americana*	Arthropoda	Malacostraca	LC50		25	4	850	[350]
Naphthalene	Opossum shrimp	*Neomysis americana*	Arthropoda	Malacostraca	LC50			4	800	[351]
Naphthalene	Opossum shrimp	*Neomysis americana*	Arthropoda	Malacostraca	LC50			4	1350	[351]
Naphthalene	Opossum shrimp	*Neomysis americana*	Arthropoda	Malacostraca	LC50			4	1250	[351]
Naphthalene	Opossum shrimp	*Neomysis americana*	Arthropoda	Malacostraca	LC50			4	1420	[351]
Naphthalene	Copepod	*Oithona davisae*	Arthropoda	Maxillopoda	LC50	Nauplii		1	4422	[230]
Naphthalene	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	LC50			4	2350	[342]
Naphthalene	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	LC50			1	2600	[340]
Naphthalene	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	LC50			2	2600	[342]
Naphthalene	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	LC50			4	2400	[352]
Naphthalene	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	LC50		21	2	2350	[353]
Naphthalene	Copepod	*Paracartia grani*	Arthropoda	Calanoida	LC50	Adult		1	2523	[230]
Naphthalene	Copepod	*Paracartia grani*	Arthropoda	Calanoida	LC50			2	2535	[229]
Naphthalene	Sea urchin	*Paracentrotus lividus*	Echinodermata	Echinoidea	LC50	Egg-pluteus		2	5588	[230]
Naphthalene	Brown shrimp	*Penaeus aztecus*	Arthropoda	Malacostraca	LC50		21	4	2500	[343]
Naphthalene	Target fish	*Terapon jarbua*	Chordata	Actinopterygii	LC50			4	15500	[349]
Naphthalene	Fiddler crab	*Uca pugilator*	Arthropoda	Malacostraca	NR-LETH		22	4	16690	[354]
Neftyany Kamni crude oil	Acorn barnacle	*Balanus improvisus*	Arthropoda	Maxillopoda	LC50			4	10300	[355]
Artem Island crude oil	Acorn barnacle	*Balanus improvisus*	Arthropoda	Maxillopoda	LC50			4	12150	[355]
Sangachaly-More crude oil	Acorn barnacle	*Balanus improvisus*	Arthropoda	Maxillopoda	LC50			4	16100	[355]
No. 2 fuel oil	Pacific rock crab	*Cancer productus*	Arthropoda	Malacostraca	TLm	Larvae	8-12	4	3560	[356]
Kuwait crude oil	Pacific rock crab	*Cancer productus*	Arthropoda	Malacostraca	TLm	Larvae	8-12	4	174800	[356]
South Louisiana crude oil	Pacific rock crab	*Cancer productus*	Arthropoda	Malacostraca	TLm	Larvae	8-12	4	218500	[356]
South Louisiana crude oil	Bamboo worm	*Capitella capitata*	Annelida	Polychaeta	LC50	Adult		2	14159	[352]
South Louisiana crude oil	Bamboo worm	*Capitella capitata*	Annelida	Polychaeta	LC50	Adult		4	10488	[352]
Cook Inlet crude oil	Pink scallop	*Chlamys hericus*	Mollusca	Bivalvia	LC50		7-10	4	2000	[231]
No. 2 fuel oil	Giant pacific chiton	*Cryptochiton stelleri*	Mollusca	Polyplacophora	TLm	Adult	4-12	4	1103.6	[337]
South Louisiana crude oil	Sheepshead minnow	*Cyprinodon variegatus*	Chordata	Actinopterygii	TLm	Adult	15-20	2	17305	[99]
No. 2 fuel oil	Sheepshead minnow	*Cyprinodon variegatus*	Chordata	Actinopterygii	TLm	Adult	15-20	4	5607	[99]

Toxicant [a]	Species common name	Species scientific name	Phylum	Class	Endpoint [b]	Life stage	Temp. (°C) [c]	Duration (days)	Conc. (µg/l) [d]	Reference
Bunker C fuel oil	Sheepshead minnow	*Cyprinodon variegatus*	Chordata	Actinopterygii	TLm	Adult	15-20	4	3029	[99]
Prudhoe Bay crude oil	Shrimp	*Eualus fabricii*	Arthropoda	Malacostraca	TLm	Larvae	5-5.5	4	5215	[338]
Prudhoe Bay crude oil	Shrimp	*Eualus fabricii*	Arthropoda	Malacostraca	TLm	Adult	3.7-10.2	4	1591	[338]
Cook Inlet crude oil	Shrimp	*Eualus fabricii*	Arthropoda	Malacostraca	TLm	Adult	4-12	1	2172	[337]
Cook Inlet crude oil	Shrimp	*Eualus fabricii*	Arthropoda	Malacostraca	TLm	Adult	4-12	4	1259	[337]
No. 2 fuel oil	Shrimp	*Eualus fabricii*	Arthropoda	Malacostraca	TLm	Adult	3.7-10.2	4	472	[338]
No. 2 fuel oil	Shrimp	*Eualus fabricii*	Arthropoda	Malacostraca	TLm	Adult	4-12	1	810	[337]
No. 2 fuel oil	Shrimp	*Eualus fabricii*	Arthropoda	Malacostraca	TLm	Adult	4-12	4	472	[338]
Cook Inlet crude oil	Mottled sea star	*Evasterias troschelii*	Echinodermata	Asteroidea	LC50			4	820	[357]
Cook Inlet crude oil	Purple shore crab	*Hemigrapsis nudus*	Arthropoda	Malacostraca	LC50			4	8450	[227]
Venezuelan Tia Juana crude oil	American lobster	*Homarus americanus*	Arthropoda	Malacostraca	LC50			4	4600	[358]
Venezuelan Tia Juana crude oil	American lobster	*Homarus americanus*	Arthropoda	Malacostraca	LC50			4	4900	[358]
Venezuelan Tia Juana crude oil	American lobster	*Homarus americanus*	Arthropoda	Malacostraca	LC50			4	860	[358]
No. 2 fuel oil	Black katy	*Katharina tunicata*	Mollusca	Polyplacophora	TLm	Adult	4-12	1	917	[337]
No. 2 fuel oil	Black katy	*Katharina tunicata*	Mollusca	Polyplacophora	TLm	Adult	4-12	4	392	[337]
No. 2 fuel oil	Plate limpet	*Notoacmea scutum*	Mollusca	Gastropoda	TLm		4-12	4	4486	[337]
Cook Inlet crude oil	Plate limpet	*Notoacmea scutum*	Mollusca	Gastropoda	TLm	Adult	4-12	4	3146	[338]
Prudhoe Bay crude oil	Plate limpet	*Notoacmea scutum*	Mollusca	Gastropoda	LC50			4	8180	[227]
Cook Inlet crude oil	Coho salmon	*Oncorhynchus kisutch*	Chordata	Actinopterygii	LC50			4	730	[359]
Cook Inlet crude oil	Coho salmon	*Oncorhynchus kisutch*	Chordata	Actinopterygii	LC50			4	1080	[359]
No. 2 fuel oil	Worm	*Ophryotrocha sp.*	Annelida	Polychaeta	LC50			4	2900	[360]
South Louisiana crude oil	Worm	*Ophryotrocha sp.*	Annelida	Polychaeta	LC50			4	12900	[360]
No. 2 fuel oil	Worm	*Ophryotrocha puerilis*	Annelida	Polychaeta	LC50			4	2200	[360]
South Louisiana crude oil	Worm	*Ophryotrocha puerilis*	Annelida	Polychaeta	LC50			4	17200	[360]
Shengli Crude Oil	Red seabream	*Pagrus major*	Chordata	Actinopterygii	LC50			2	6400	[361]
Neftyany Kamni crude oil	Shrimp	*Palaemon elegans*	Arthropoda	Malacostraca	LC50			4	2350	[355]
Artem Island crude oil	Shrimp	*Palaemon elegans*	Arthropoda	Malacostraca	LC50			4	3150	[355]
Sangachaly-More crude oil	Shrimp	*Palaemon elegans*	Arthropoda	Malacostraca	LC50			4	6800	[355]
No. 2 fuel oil	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	TLm	Adult	21	2	4895	[353]
Bunker C fuel oil	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	TLm	Adult	21	2	3351	[353]
South Louisiana crude oil	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	TLm	Adult	24	2	13125	[353]
South Louisiana crude oil	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	TLm	Adult	32	2	8945	[353]
No. 2 fuel oil	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	LC50			1	4400	[99]
No. 2 fuel oil	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	LC50			2	4100	[99]

Toxicant [a]	Species common name	Species scientific name	Phylum	Class	Endpoint [b]	Life stage	Temp. (°C) [c]	Duration (days)	Conc. (µg/l) [d]	Reference
Bunker C residual	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	LC50			1	3200	[99]
Bunker C residual	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	LC50			2	2800	[99]
Bunker C residual	Daggerblade grass shrimp	*Palaemonetes pugio*	Arthropoda	Malacostraca	LC50			4	2600	[99]
Cook Inlet crude oil	Pandalid shrimp	*Pandalus danae*	Arthropoda	Malacostraca	TLm	Adult	4-12	1	819	[337]
Cook Inlet crude oil	Pandalid shrimp	*Pandalus danae*	Arthropoda	Malacostraca	TLm	Adult	4-12	4	698	[337]
No. 2 fuel oil	Pandalid shrimp	*Pandalus danae*	Arthropoda	Malacostraca	TLm	Adult	4-12	1	1495	[337]
No. 2 fuel oil	Pandalid shrimp	*Pandalus danae*	Arthropoda	Malacostraca	TLm	Adult	4-12	4	988	[337]
Cook Inlet crude oil	Coonstripe shrimp	*Pandalus hypsinotus*	Arthropoda	Malacostraca	LC50		7-10	4	1400	[231]
Prudhoe Bay crude oil	Coonstripe shrimp	*Pandalus hypsinotus*	Arthropoda	Malacostraca	TLm	Adult	3.7-10.2	4	1607	[338]
Prudhoe Bay crude oil	Coonstripe shrimp	*Pandalus hypsinotus*	Arthropoda	Malacostraca	TLm	Larvae	5-5.5	4	6995	[338]
Cook Inlet crude oil	Coonstripe shrimp	*Pandalus hypsinotus*	Arthropoda	Malacostraca	TLm	Adult	4-12	1	2474	[337]
Cook Inlet crude oil	Coonstripe shrimp	*Pandalus hypsinotus*	Arthropoda	Malacostraca	TLm	Adult	4-12	4	2345	[337]
Shengli crude oil	Bastard halibut	*Paralichthys olivaceus*	Chordata	Actinopterygii	LC50			4	1600	[361]
Bunker C fuel oil	Brown shrimp	*Penaeus aztecus*	Arthropoda	Malacostraca	TLm	Adult	15-20	2	3420	[99]
Bunker C fuel oil	Brown shrimp	*Penaeus aztecus*	Arthropoda	Malacostraca	TLm	Adult	15-20	4	1856	[99]
Shengli crude oil	Fleshy prawn	*Penaeus chinensis*	Arthropoda	Malacostraca	LC50			4	510	[361]
Shengli crude oil	Fleshy prawn	*Penaeus chinensis*	Arthropoda	Malacostraca	LC50			4	9000	[361]
Cook Inlet crude oil	Long-jaw flounder	*Platichthys stellatus*	Chordata	Actinopterygii	LC50		7-10	4	1800	[231]
South Louisiana crude oil	Dumeril's clam worm	*Platynereis dumerilii*	Annelida	Polychaeta	LC50	Adult		2	10283	[352]
South Louisiana crude oil	Dumeril's clam worm	*Platynereis dumerilii*	Annelida	Polychaeta	LC50	Adult		4	7942	[352]
No. 2 fuel oil	Northern kelp crab	*Pugettia producta*	Arthropoda	Malacostraca	TLm	Larvae	8-12	4	8900	[356]
Kuwait crude oil	Northern kelp crab	*Pugettia producta*	Arthropoda	Malacostraca	TLm	Larvae	8-12	4	437000	[356]
South Louisiana crude oil	Northern kelp crab	*Pugettia producta*	Arthropoda	Malacostraca	TLm	Larvae	8-12	4	376200	[356]
Neftyany Kamni crude oil	Harris mud crab	*Rhithropanopeus harrisii*	Arthropoda	Malacostraca	LC50			4	22820	[355]
Artem Island crude oil	Harris mud crab	*Rhithropanopeus harrisii*	Arthropoda	Malacostraca	LC50			4	25100	[355]
Sangachaly-More crude oil	Harris mud crab	*Rhithropanopeus harrisii*	Arthropoda	Malacostraca	LC50			4	28140	[355]
Cook Inlet crude oil	Manila clam	*Tapes semidecussata*	Mollusca	Bivalvia	LC50			4	5210	[362]

[a] Toxicity values for oil resulted from experiments with water-soluble fractions (WSF).
[b] TLm values were converted from ppm (µl/l) to mg/l with the densities of No. 2 (0.89 mg/µl) and Bunker C fuel oil (0.98 mg/µl), Prudhoe bay (0.82 mg/µl), Cook Inlet (0.86 mg/µl), S. Louisiana (0.84 mg/µl), and Kuwait crude oil (0.87 mg/µl).
[c] Temperature (˚C)
[d] Experimental concentration (µg/l)

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Summary

In ecological risk assessment (ERA) models can be used to predict chemical exposure and effects on aquatic species and ecosystems. The key question in this thesis is whether we can use simple, generic models to assess the risk of chemical pollutants in specific systems, such as oil constituents in the Arctic ecosystem (**chapter 1**). The aim of this PhD thesis was therefore to evaluate various chemical exposure and effect models on their applicability in ERA for aquatic species and ecosystems, with a focus on oil constituents. Models were evaluated for different steps in ERA: bioaccumulation, single-species effects and multi-species effects. Most of the model evaluation was done based on crude oil constituents. A consumer-resource population model was evaluated based on an experimental study with a herbicide instead, because the more specific toxicity of the herbicide (i.e. primarily affecting the resource species) better facilitated investigation of indirect chemical effects (i.e. by affecting food availability for the consumer species).

Chemical concentrations in an organism (body burdens; BB) can be derived from measurements and by using bioaccumulation models that can estimate internal concentrations based on kinetic parameters, e.g. absorption and elimination rate constants. Although several bioaccumulation models have been developed, few have been used to quantify the accumulation of oil constituents in aquatic species. The aim of **chapter 2** was to evaluate the applicability of a generic toxicokinetic model (OMEGA) for estimating oil constituent accumulation in aquatic species. To this end, model predictions of absorption and elimination rate constants and bioconcentration factors (BCF) were compared with laboratory-derived measurements collected from the literature. The dataset included aquatic species from different taxonomic groups (Annelida, Chlorophyta, Crustacea, Insecta, Mollusca and Osteichthyes) exposed to constituents from different oil groups (mono-, di-, and polycyclic aromatic hydrocarbons, phenols and n-paraffins). The model performance was evaluated using the coefficient of efficiency (E) and the root-mean-square-error (RMSE). It was found that the difference between individual measurements and the estimated absorption and elimination rate constants from the OMEGA model was smaller than the difference between the individual measurements and the means of the measurements. However, the OMEGA model did not outperform the mean measured BCF. More specifically, modelled absorption and elimination rates were reasonably accurate for crustaceans, but much less so for fish. It was also shown that correcting absorption rate constants for molecular mass may improve model performance for oil constituents with a relatively low mass (e.g. mono- and dicyclic aromatic hydrocarbons). Furthermore, the addition of biotransformation as an additional elimination route will improve model estimations for taxonomic groups able to biotransform oil constituents, such as Osteichthyes (bony fish).

In **chapter 3** the OMEGA model was evaluated in a dynamic context by quantifying the time-varying effects of eight aromatic hydrocarbons (oil constituents) on the survival of four crustacean and three fish species. First, the body burdens of the oil constituents in aquatic organisms were estimated over time. Next, survival

was assumed to be a log-logistic function of the body burden in order to estimate the toxic impact of the oil constituents on aquatic organisms. The model was based on key parameters applicable to an array of species and compounds with baseline toxicity (narcosis) reflected by an average lethal body burden (LBB). Finally, the model results for eight oil constituents were compared with measured effects to evaluate the overall goodness-of-fit of the model. Generally, the survival of crustaceans and fish as estimated by the OMEGA model was lower than the measured survival right after the onset of oil constituent exposure in the laboratory. In contrast, the model underestimated the maximum mortality over time. Differences were most likely induced by assumptions on the constant LBB and the time required to arrive at steady state concentrations of the oil constituents in organisms. A more complex model approach is most likely needed to predict toxicity dynamics of narcotic chemicals.

Marine copepods constitute an important component of (sub) arctic marine food webs. Until now, effects of crude oil on copepod biomass in the subarctic have not been estimated simultaneously by population models of different complexity. The goal of **chapter 4** was therefore to evaluate the difference between the multistage SINMOD population model and a simpler consumer-resource population model (Rosenzweig-MacArthur) for estimating the effects of crude oil on the copepod *Calanus finmarchicus*, which plays an important role in subarctic marine ecosystems. The OMEGA model was used to estimate effects of a simulated hypothetical oil spill on the copepods' survival and reproduction (used as input to SINMOD) or on copepod survival only (as input to the Rosenzweig-MacArthur model). Both population models showed a negligible effect of crude oil on the species' biomass assuming low species sensitivity to crude oil. When assuming moderate to high species sensitivity, the simple model estimated a larger impact of crude oil on the biomass compared to the complex model. The total internal concentrations of crude oil in *C. finmarchicus* (total body burdens; TBBs) estimated by the simple model were on average a factor of 3 higher than those estimated by the complex model, thereby causing higher estimated mortality rates. This difference in TBBs may be related to the inclusion of the advection of copepods in space and time in the complex model, resulting in the dilution of TBBs. The possibility of including a copepod advection rate affecting the time-varying biomass should be examined to improve the estimates of the simple consumer-resource model.

The objective of **chapter 5** was to use a generic population model to explore consumer-resource dynamics in a marine ecosystem. To this end, short-term experiments were combined with population modelling to explore long-term effects of the herbicide atrazine on marine consumer-resource dynamics. The 28-day lab experiments indicated that the intrinsic rate of increase (r) and carrying capacity (K) of the marine diatom *Seminavis robusta* decreased with increasing atrazine exposure. These experimentally observed atrazine-induced decreases of r and K were used to parameterize a Rosenzweig-MacArthur population model representing a simple food chain including the tested diatom and its

grazer, the harpacticoid copepod *Delavalia palustris* var. *palustris*. Thus, the population model was used for estimating indirect effects of toxic stress via the food availability on the copepod. Stable oscillating consumer-resource systems were produced at diatom exposures of 0, 100 and 150 µg/L atrazine. An atrazine concentration of 150 µg/L resulted in a 15% increase of the oscillation periods of both diatoms and copepods and a 52% reduction of diatom density oscillation amplitudes compared to the control situation. The maximum and minimum copepod density reduced by 61% and 63%, respectively. Although the consumer-resource model is simpler than more detailed marine food-web models and its predictions need to be tested using field data, the simulations suggested food chain-mediated indirect effects of toxicants on zoobenthos populations, indicating reduced diatom and copepod availability throughout the year.

Toxicity data provide an important component of ecological risk assessments. Until now, risk assessments for the Arctic have typically been based on toxicity data obtained for species from temperate regions, without knowing if these toxicity data are representative. In **chapter 6**, sensitivities of polar and temperate marine species to crude oil, 2-methyl-naphthalene and naphthalene were compared by using Species sensitivity distributions (SSDs). An SSD is a cumulative distribution curve which is based on toxicity data of multiple species within a region and therefore represents inter-species variation in sensitivity. In chapter 6, SSDs were based on toxicity data which comprised acute LC_{50}, EC_{50} and TL_m (median tolerance limit) endpoint values, with mortality or reduced survival effects for 50 percent of the test organisms. Between the two species groups, there was a maximum factor of 3 difference in sensitivity to crude oil and oil constituents, based on the means of the toxicity data and the hazardous concentrations for 5 and 50 percent of the species (HC_5 and HC_{50}) as derived from the SSDs. Polar and temperate species sensitivities generally did not significantly differ from each other. Physiological mechanisms that have been suggested to cause differences in sensitivity between polar and temperate marine species, such as lipid composition, antioxidant levels and resistance to freezing, apparently have only little impact on species' sensitivity to the oil constituents investigated here. Based on the oil constituents and mixtures in chapter 6, toxicity data from temperate species may be used in risk assessment for Arctic species in data scarce situations.

In **chapter 7**, the general key question of this thesis was addressed by evaluating a set of assumptions, each representing a possible simplification in exposure and effect modelling employed to assess risks of chemicals in aquatic ecosystems. It was concluded that a model for risk assessment of crude oil constituents has to include uptake via water, biotransformation factors for fish and possibly for crustaceans, and a simplified migration process. There are no indications to differentiate between temperate and polar species in constructing an SSD for oil constituents. Furthermore, trophic bottom-up effects of organic chemicals are probably only important for risk assessment of chemicals with a specific toxicity on basal species such as phytoplankton, for example herbicides (i.e. atrazine),

and not for narcotic chemicals with a generic baseline toxicity. It is probably not necessary to include CBBs for toxic modes of action other than narcosis when crude oil is divided into several hydrocarbon blocks. Nevertheless, a single CBB for narcosis is not always applicable when assessing risks for individual crude oil constituents. Some constituents exhibit a more specific toxicity than narcosis and the assumption of a single CBB at different exposure times is probably too generic.

Several improvements are suggested for future usage of the generic and simple bioaccumulation, effect and population models evaluated in this thesis. Firstly, it is recommended to add biotransformation rates for fish to the OMEGA model. Secondly, it is recommended to further investigate the validity of the CBB concept for the effect assessment of crude oil constituents. This includes whether multiple CBBs should be produced that are distinctive between oil constituents with dissimilar TMoAs and between different exposure times. Thirdly, it is recommended to include a migration factor in simple population models that are used for ERA of crude oil. Finally, when more data become available, exceptions of species sensitivity differences could also be tested using SSDs based on acute and chronic external and internal toxicity data for more oil constituents, species, and different organism life stages.

Samenvatting

In een ecologische risicobeoordeling van chemische stoffen wordt vaak gebruik gemaakt van computermodellen om de blootstelling van en effecten op aquatische soorten en ecosystemen in te schatten. De hoofdvraag in dit proefschrift is of generieke blootstellings- en effectmodellen een adequate inschatting kunnen geven van het risico van milieuverontreinigende stoffen in specifieke gebieden, in het bijzonder van oliecomponenten in het Arctische ecosysteem (**hoofdstuk 1**). Daartoe zijn modellen geëvalueerd voor verschillende aspecten van risicobeoordeling: bioaccumulatie, effecten op één soort en effecten op meerdere (elkaar beïnvloedende) soorten. Alle modelevaluaties zijn uitgevoerd op basis van ruwe oliecomponenten, met uitzondering van een predator-prooi populatiemodel dat op basis van een experimentele studie met een herbicide is geëvalueerd. De specifieke toxiciteit van de herbicide voor de prooi vereenvoudigde namelijk het onderzoek naar indirecte chemische effecten via de voedselbeschikbaarheid voor de predator.

Het doel van **hoofdstuk 2** was om de toepasbaarheid te evalueren van een generiek toxicokinetisch model (OMEGA) voor het schatten van de accumulatie van oliecomponenten in aquatische soorten. Hiertoe werden modelschattingen van absorptie- en eliminatiesnelheidsconstanten en bioconcentratiefactoren vergeleken met in de literatuur gerapporteerde meetgegevens voor aquatische organismen (waaronder beenvissen, groenwieren, insecten, kreeftachtigen, ringwormen, weekdieren) blootgesteld aan componenten van verschillende oliegroepen (mono-, di-, en polycyclische aromatische koolwaterstoffen, fenolen en n-paraffines). Het bleek dat de afwijking tussen de individuele meetgevens en de voorspelde absorptie- en eliminatiesnelheidsconstanten van het OMEGA model kleiner was dan de afwijking tussen de individuele meetgevens en het gemiddelde van alle meetgegevens. Dit gold niet voor de biococentratiefactoren. Gemodelleerde absorptie- en eliminatiesnelheidsconstanten waren nauwkeuriger voor kreeftachtigen dan voor beenvissen. Een correctie van de absorptiesnelheidsconstanten op basis van moleculaire massa kan het model verbeteren voor oliecomponenten met een relatief lage moleculaire massa (bijvoorbeeld mono- en dicyclische aromatische koolwaterstoffen). Daarnaast verbetert de toevoeging van biotransformatie als een aanvullende eliminatieroute de modelschattingen voor organismen die in staat zijn om oliecomponenten te transformeren, zoals beenvissen.

In **hoofdstuk 3** werd het OMEGA model in een dynamische context getest door tijdsafhankelijke effecten van acht aromatische koolwaterstoffen op de overleving van vier kreeftachtigen en drie vissoorten te bepalen. Allereerst werden tijdsafhankelijke interne concentraties van de oliecomponenten in aquatische organismen geschat. Vervolgens werd op basis van een log-logistische functie van de interne concentraties het toxische effect van de oliecomponenten op de overleving van aquatische organismen geschat. De modelschattingen zijn ten slotte vergeleken met gemeten effecten uit laboratoriumstudies. De gemodelleerde overleving van kreeftachtigen en vissen bleek lager te zijn dan de gemeten overleving direct na het begin van de blootstelling. Aan het einde

van de blootstellingsperiode overschatte het OMEGA model echter de overleving van de organismen. Mogelijke verklaringen voor verschillen tussen schattingen en metingen zijn de modelaanname van een constante drempelwaarde voor het optreden van effecten en de aanname dat concentraties van oliecomponenten snel een vaste verhouding tussen het water en de organismen bereiken. Om de effecten van oliecomponenten over de tijd te voorspellen is hoogstwaarschijnlijk een meer complexe modelbenadering nodig.

Mariene roeipootkreeften, zoals *Calanus finmarchicus*, maken een belangrijk onderdeel uit van het mariene (sub)arctische voedselweb. Om de effecten van ruwe olie op de biomassa van roeipootkreeften te kwantificeren zijn verschillende populatiemodellen beschikbaar. Het doel van **hoofdstuk 4** was om de verschillen te evalueren in de schatting van de effecten van ruwe olie op de roeipootkreeft C. finmarchicus door het complexe populatiemodel SINMOD versus het simpelere predator-prooi populatiemodel Rosenzweig-MacArthur. Bij de aanname van een lage gevoeligheid van de soort voor ruwe olie toonden beide populatiemodellen verwaarloosbare effecten van ruwe olie op de biomassa van *C. finmarchicus*. Bij een gemiddeld tot hoog veronderstelde soortgevoeligheid schatte het simpele model een groter effect van ruwe olie op de biomassa ten opzichte van het complexe model. De geschatte interne concentraties van ruwe olie in *C. finmarchicus* van het simpele model waren gemiddeld een factor 3 hoger dan die van het complexe model. Het verschil werd veroorzaakt doordat het complexe model de migratie van de roeipootkreeften in ruimte en tijd meeneemt, waardoor de interne concentraties lager uitvielen. Om de schattingen van het simpele predator-prooi populatiemodel te verbeteren is het aan te raden om de invloed van migratie op de tijdsafhankelijke veranderingen in biomassa mee te nemen in de berekeningen.

Het doel van **hoofdstuk 5** was om de populatiedynamiek van een predator en zijn prooi in een marien ecosysteem te onderzoeken met behulp van het algemene populatiemodel Rosenzweig-MacArthur. Resultaten van relatief kortdurende experimenten werden gecombineerd met populatiemodellering om lange-termijn effecten van de onkruidverdelger atrazine op de populatiedynamiek van een mariene predator (roeipootkreeft) en prooi (diatomee) te bestuderen. De laboratorium experimenten duurden 28 dagen en toonden aan dat de intrinsieke groeisnelheid (r) en draagkracht (K) van de mariene diatomeeënsoort *Seminavis robusta* afnamen met toenemende blootstelling aan atrazine. Vervolgens werden deze observaties gebruikt om het Rosenzweig-MacArthur populatiemodel te parameteriseren voor de geteste soort en zijn predator, de roeipootkreeft *Delavalia palustris* var. *palustris*. Dit maakte het mogelijk om via de voedselbeschikbaarheid indirecte effecten van atrazine op de roeipootkreeft te kwantificeren. Bij de hoogste atrazineconcentratie (150 µg/L) daalde de dichtheid van de diatomeeën met 52% in vergelijking met de controle. De dichtheid aan roeipootkreeften nam af met 61-63%. Deze simulaties tonen aan dat indirecte effecten van toxische stoffen via de voedselketen kunnen worden overgebracht.

Toxiciteitsdata vormen een belangrijk onderdeel van een ecologische risicobeoordeling van een stof. Risicobeoordelingen voor het Arctische gebied worden doorgaans gebaseerd op toxiciteitsdata voor soorten uit gematigde streken, zonder te weten of deze data representatief zijn voor koudere omstandigheden. In **hoofdstuk 6** werd de gevoeligheid van mariene soorten uit poolgebieden en gematigde gebieden voor ruwe olie, 2-methylnaftaleen en naftaleen vergeleken met behulp van soortgevoeligheidsverdelingen (Species sensitivity distributions; SSDs) op basis van acute toxiciteitsdata. Een SSD is een cumulatieve verdelingscurve die gebaseerd is op toxiciteitsdata van meerdere soorten in een gebied en daarmee de variatie in gevoeligheid tussen soorten weergeeft. De vergelijking van de SSDs toonde aan dat de gevoeligheid voor ruwe olie en oliecomponenten maximaal een factor 3 verschilde tussen de gematigde en Arctische soorten. In de meeste gevallen was het verschil niet significant. Fysiologische mechanismen die voorheen als oorzaak werden gezien voor de verschillen in gevoeligheid tussen mariene poolsoorten en gematigde soorten, zoals vetsamenstelling, antioxidantengehalte en weerstand tegen bevriezing, hebben mogelijk weinig effect op de soortgevoeligheid voor de oliecomponenten die in deze studie zijn onderzocht. Dit suggereert dat toxiciteitsdata van gematigde soorten gebruikt kunnen worden in de risicobeoordeling van oliecomponenten voor Arctische soorten.

In **hoofdstuk 7** werd op basis van de verkregen onderzoeksresultaten geconcludeerd dat effecten van lage naar hogere trofische niveaus ('bottom-up') waarschijnlijk alleen belangrijk zijn voor de risicobeoordeling van chemische stoffen met een specifieke toxiciteit voor fundamentele soorten als fytoplankton, bijvoorbeeld herbiciden zoals atrazine, en niet voor stoffen met een narcotische werking. Daarnaast werd geconcludeerd dat er geen aanwijzingen zijn om onderscheid te maken tussen mariene gematigde soorten en poolsoorten voor het samenstellen van een SSD voor oliecomponenten. Vervolgens zijn verschillende verbeteringen voorgesteld voor de generieke bioaccumulatie-, effect- en populatiemodellen die zijn geëvalueerd in dit proefschrift. Allereerst is het raadzaam om biotransformatiesnelheden van vissen toe te voegen aan het OMEGA model. Ten tweede wordt aanbevolen om bij de beoordeling van effecten van ruwe oliecomponenten de invloed van blootstellingsduur verder te onderzoeken. Ten derde wordt aanbevolen om een migratiefactor mee te nemen in simpele populatiemodellen die worden gebruikt voor de ecologische risicobeoordeling van ruwe olie. Ten slotte, wanneer meer data beschikbaar komt, kunnen uitzonderingen in verschillen in soortgevoeligheid ook worden onderzocht met SDDs op basis van acute en chronische toxiciteitsdata voor meer oliecomponenten, soorten en levenstadia van organismen.

Acknowledgements
Dankwoord

Het schrijven van dit dankwoord voelt als een plechtig moment. Het zijn mijn laatste woorden in dit proefschrift en ik krijg eindelijk de kans om de mensen te bedanken die de afgelopen jaren zoveel voor mij hebben betekend.

Allereerst wil ik Jan, Mark, Aafke en Frederik bedanken voor de bijdrage die zij hebben geleverd als begeleiders tijdens mijn promotietraject. Jullie hebben mij de afgelopen jaren onder jullie hoede genomen, waarvoor mijn grootste dank. Ik heb ontzettend veel geleerd en onze samenwerking heeft uiteindelijk tot dit mooie resultaat geleid. Iets om trots op te zijn!

Jan, bedankt dat je potentie in mij zag en dit bleef zien door de jaren heen. Je deur stond voor mij open om me weer op weg te helpen wanneer ik vast zat. Ik ben de tel kwijtgeraakt van het aantal gesprekken die wij hebben gevoerd over SSDs, OMEGA en SINMOD. Je praktische instelling heeft mij meer dan eens vooruit geholpen, zowel in mijn werkzaamheden als privé. Ik hoop van harte dat je gezondheid snel verbetert.

Mark, jouw kritische houding en onuitputbare ideeën stelden mij bij elk overleg weer op scherp. Je hebt me meegegeven om me staande te houden en mijn mening te ventileren, iets waar ik altijd profijt van zal hebben. Aan het begin van dit jaar heb je me ondersteund toen ik het nodig had. Enorm bedankt. De laatste loodjes wegen het zwaarste, lekker cliché maar daarom niet minder waar.

Aafke, de onverminderde energie waarmee je je op mijn werk stortte, bewonder ik zeer. Je was altijd bereid om mee te denken, wat ik zeer fijn vond. Je oplossingen en zorgvuldige reviews droegen bij aan een betere kwaliteit van mijn papers, ook al betekende dit soms het aanpassen van mooie rood gekleurde teksten ;-)

Frederik, sinds ik vijf jaar geleden startte met mijn masterstage in Gent, ben je al betrokken bij mijn onderzoek en daar ben ik blij om. De afgelopen jaren heb ik je vooral gezien tijdens bijeenkomsten in het buitenland, maar via de mail hadden we goed contact en via skype kon ik gelukkig met je sparren over R en populatiemodellering. Je hebt altijd een nuttige bijdrage geleverd aan mijn onderzoek, bedankt daarvoor.

Voor het SYMBIOSES project heb ik samengewerkt met sympathieke mensen die ik geregeld heb mogen ontmoeten op de mooiste locaties in Noorwegen. Karel, je bent een toffe collega die zelfs zijn humor en kalmte weet te behouden bij een stressvol moment op een berg. Je ben een goede onderzoeker en ik ben ervan overtuigd dat je een goede toekomst tegemoet gaat. Ole Jacob, thank you for all the hours you've spent on (re)running the model and answering my many questions. Although the situation wasn't always ideal, we did the job! JoLynn, Chris, Lindsey, and Jonas, thank you for the pleasant collaboration during the course of the project and for the interesting assemblies in Norway. Chris and Lindsey, we will always think twice where to put our feet if we walk on a mountain or down the streets in Norway ;-) Mathijs, bedankt dat je mij als

masterstudent de kans hebt gegeven om mijn bacheloronderzoek verder uit te werken tot een volwaardige paper en mij uit te nodigen bij Statoil in Trondheim. Fijn dat je mijn verdediging van zo dichtbij wil meemaken.

Voordat ik begon met mijn werkzaamheden voor het SYMBIOSES project was ik bezig met het afronden van mijn stage in het lab in Gent en achter de pc in Nijmegen. Uiteindelijk is dat werk een hoofdstuk geworden van dit proefschrift; iets wat nooit mogelijk was geweest zonder de hulp van Jan, Frederik, Marleen, Clio, Nancy en de medewerkers van de afdeling mariene biologie en het GhEnToxLab in Gent. Deze samenwerking tussen noorder- en zuiderburen was geslaagd en zal ik niet vergeten.

Collega's van milieukunde, jullie wil ik bedanken voor het geven van luchtigheid aan serieuze zaken en in het voorzien van mijn behoefte om te kletsen. Natuurlijk mijn roommates: Francesca, Gea, Joris, Katja, Laura V, Leonie, Rascha, Renske, Sadegh, Tom, Valerio, Yen en Zoran. De guys next door: Ana, Anne, Alessandra, Daan, Frank, Isabel, Jelle, Jon, Laura G, Ligia, Mélanie, Pieter, Remon, Rik, Steef, Wan Yee en Zahar. En natuurlijk: Ad, Bor, Gina, Heleen, Karin, Marianne, Marieke en Rosalie. Rob, bedankt voor alle goede adviezen en bedankt dat je me hebt betrokken bij het interessante onderzoek naar invasieve exoten. Hopelijk duurt onze samenwerking nog even voort. Laura V, bedankt voor de vele pauzes en leuke samenwerking. Als je hulp nodig hebt in Zweden dan roep je maar, ik kom je graag opzoeken.

Collega's bij Stichting Bargerveen, fijn dat jullie mij opnemen in de groep, ook al ben ik vooral een theoreticus ;-) Janneke, je staat nog aan het begin van je promotietraject, maar ik heb het volste vertrouwen dat jouw vastberadenheid en passie je tot de eindstreep zullen brengen.

In het bijzonder wil ik mijn paranimfen bedanken.

Thomas, in de afgelopen 9 jaar hebben we vanalles meegemaakt, van lopend over de catwalk tot banjerend door het Swin en liftend naar Luxemburg tot dansend bij de Chemical Brothers. Uiteindelijk werden we collega's en bleven we goede herinneringen maken, zowel op als buiten de afdeling. Ik vind het fijn om te weten dat we alles kunnen delen, verdrietige en euforische momenten, en dat moeten we vooral blijven doen.

Stephanie, je neemt mij altijd mee in je enthousiasme en gekkigheid en daar hou ik van. Het is nooit saai met jou, al zitten we met panne in een Indiaas gehucht of staan we in de regen op de markt met een opblaasband! Gelukkig kunnen we ook bij elkaar terecht voor PhD gerelateerde zaken, twee mensen dragen meer dan één. Ik kijk al uit naar ons volgende decennium met dwaze en memorabele momenten.

Met mijn vrienden heb ik de laatste jaren gelukkig ook lief en leed kunnen delen. Jela, we hebben samen intense en rustigere jaren meegemaakt maar ik heb altijd op je kunnen bouwen. We moeten ons 10-jarig jubileum maar eens goed vieren. Jessie, ik bewonder je doorzettingsvermogen en bedank je voor de afleiding toen ik het nodig had. Roel, super bedankt voor je hulp bij de afronding van dit boek, zonder jou was het niet zo mooi geworden. Annet, Hanna, Ilse, Jozette en Vicky, ook al woon ik alweer 10 jaar in Nijmegen, dankzij jullie heb ik gelukkig nog geregeld contact met mijn Zeeuwse roots. Mede-PhDers Nils, Eva en Merit, succes met de afronding van jullie onderzoek, hopelijk volgen er nog veel feestjes en festivals. Maartje en Marjolein, een dansje met jullie hielp altijd goed tegen stress, daar wil ik graag gebruik van blijven maken. Martijn, nu we praktisch buren zijn, kan het hebben van een stamkroeg in Oud-West eigenlijk niet meer uitblijven. Iris, vergeleken met de afstand die je lopend hebt afgelegd, is zo'n promotieonderzoek best goed te doen, succes!

Marcel, ook al wonen we niet bij elkaar in de buurt, ik wil je bedanken dat je me als oudere broer een luisterend oor bood toen ik het nodig had. Soms loopt het leven anders dan je hebt gedacht maar wat er ook gebeurt, ik sta altijd voor je klaar. Misschien kunnen we wel zo goed met elkaar overweg, omdat we toch meer op elkaar lijken dan we denken.

Lieve pa en ma, jullie hebben de basis gelegd voor al die jaren dat ik in Nijmegen heb geleerd en gewerkt. Jullie hebben me altijd vrij gelaten in het maken van keuzes, waardoor ik mij kon ontwikkelen tot wie ik nu ben. Bedankt voor jullie onvoorwaardelijke steun tijdens mijn promotietraject. Ik was nooit zover gekomen zonder jullie. En ma, niet huilen hoor, dan moet ik ook.

Lieve Lennart, je weet niet half hoeveel je voor mij betekent. Je bent de stabiele factor geweest in de turbulente laatste jaren, mede door je immer aflatende optimisme en geduld. Met je bijna Zeeuwse nuchterheid relativeer je moeilijke zaken en vanwege je Brabantse humor lach ik elke dag. Ik prijs mij ontzettend gelukkig dat ik je ken en weet zeker dat wij een mooie tijd tegemoet gaan!

About the author

Curriculum vitae

On August 22nd 1988 I was born in Vlissingen, the Netherlands. I grew up in Westkapelle and attended secondary school in Middelburg (Nehalennia SSG). After my graduation in 2006, I moved to Nijmegen to study Biology at the Radboud University. I started to work at the Department of Environmental Science during my bachelor's internship, in which I researched the sensitivity of aquatic species to toxic substances. In 2009 I finished my bachelor's degree with a specialization in Environmental Science ('bene meritum'). I continued with a master's programme in Environmental Science for which I did an internship at B-WARE Research Centre in Nijmegen, where I participated in biogeochemical and ecohydrological research projects. For my second internship I spent three months in Gent, Belgium where I performed research on toxic stress in an aquatic predator-prey system. In between these two internships I worked as a junior researcher for Statoil on a scientific paper as a follow-up of my bachelor's thesis. In 2012 I obtained the master's degree ('bene meritum'). In the same year I started working as a junior researcher at the Department of Environmental Science. I worked for the SYMBIOSES project on bioaccumulation and effect modelling of aquatic species exposed to crude oil constituents. I had the opportunity to combine the results of my master's programme and the results obtained as a junior researcher towards a full PhD thesis. Since 2015 I am working as a researcher on projects related to the management of invasive exotic species in Europe at the department of Environmental Science and Stichting Bargerveen.

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